

Soybeans as Functional Foods and Ingredients

Editor

KeShun Liu, Ph.D.
University of Missouri
Columbia, Missouri



Champaign, Illinois

AOCS Mission Statement

To be the global forum for professionals interested in lipids and related materials through the exchange of ideas, information science, and technology.

AOCS Books and Special Publications Committee

M. Mossoba, Chairperson, U.S. Food and Drug Administration, College Park, Maryland
R. Adlof, USDA, ARS, NCAUR, Peoria, Illinois
J. Endres, The Endres Group, Fort Wayne, Indiana
T. Foglia, USDA, ARS, ERRC, Wyndmoor, Pennsylvania
L. Johnson, Iowa State University, Ames, Iowa
H. Knapp, Deaconess Billings Clinic, Billings, Montana
A. Sinclair, RMIT University, Melbourne, Victoria, Australia
P. White, Iowa State University, Ames, Iowa
R. Wilson, USDA, REE, ARS, NPS, CPPVS, Beltsville, Maryland

Copyright © 2004 by AOCS Press. All rights reserved. No part of this book may be reproduced or transmitted in any form or by any means without written permission of the publisher.

The paper used in this book is acid-free and falls within the guidelines established to ensure permanence and durability.

Library of Congress Cataloging-in-Publication Data

Soybeans as functional foods and ingredients / editor KeShun Liu.

p. cm.

Includes index.

ISBN 1-893997-33-2 (alk. paper)

1. Soybean. 2. Soybean—Composition. 3. Soybean products. 4. Soyfoods.

I. Liu, KeShun, 1958-

SB205.S7S554 2005

633.3'4—dc22

2004016292

Printed in the United States of America

08 07 06 05 04 5 4 3 2 1

Preface

A few thousand years ago, soybeans originated and were cultivated as a food crop in China. For thousands of years, this Oriental treasure was a well-kept secret of the region. Large-scale introduction of soybeans to the West did not begin until the beginning of the 20th century. Since then, much progress has been made with respect to the cultivation, production, processing, and end use applications of soybeans, mainly due to technological innovations and improvements in our understanding of soybean chemistry. This recent revolution in soybean production and end use processing has led to a rapid increase in soybean production on a global basis and to development of various new uses of soybeans as food, feed, and industrial materials. World production has reached 180 million tons annually and continues to increase.

The soybean is unique in that it contains 40% protein with all essential amino acids and 20% oil. As a food, it is nutritious. Yet, traditional soyfoods developed in China and neighboring countries thousands of years ago have less appeal to the Western population due to their unfamiliar taste and texture. As a result, the majority of soybean production is crushed into oil and defatted meal. Although the oil is used mainly in edible applications, the defatted meal is used largely as animal feed. Only a small portion is processed into food protein ingredients. Clearly, we need another revolution to reverse this situation.

Fortunately, a new revolution has in fact begun since the late 20th century. For many years, soybeans have been primarily identified with their high protein and oil content. Yet, for the past decade, there has been much interest among medical researchers in elucidating the health benefits of direct human consumption of soybeans as food. Mounting evidence indicates that regular consumption of soyfoods can reduce the incidence of breast, colon, and prostate cancers; prevent heart disease and osteoporosis; and alleviate menopausal symptoms. Among the many soy components examined, isoflavones and soy proteins exhibit the most promise as key components responsible for the health benefits of soy. Soy is unique in that it contains as much as four milligrams of isoflavones per gram of dry matter, whereas cereals and other legumes contain almost none. Other components under investigation for their roles in the health effects of soy include saponins, lecithin, phytosterols, phytate, trypsin inhibitors, and oligosaccharides. Some of these were originally thought to be antinutrients.

In response to the medical research, in late 1999, the U.S. Food and Drug Administration approved a health claim regarding the cholesterol-lowering effect of soy protein. Medical discovery about the health benefits of soy and the FDA ruling have set off a rush by mainstream food companies to enter the soyfoods market. This has helped to increase the awareness of soyfood products, turning the image of soy from negative to positive. It has also created an incentive for food processors to incorporate soy protein ingredients into many types of existing foods. Countless new medical studies about soy health benefits are continuously being undertaken, and a new petition about cancer prevention of soy is currently under FDA review.

Coupled with this new revolution in soybean research is our growing interest in the relationship between diet and health. In modern society, we have turned to drugs to treat or prevent diseases. However, since the discovery of nutrients and our increasing analytical capabilities at the molecular level, we are beginning to become more knowledgeable of the biochemical structure-function relationships of the myriad chemicals that occur naturally in foods and their effects on the human body. This has spawned a whole new industry since the later years of the last century—functional foods.

Functional foods, designer foods, and nutraceuticals are terms used interchangeably to refer to foods or food components that can provide physiological benefits by enhancing overall health, including the prevention and treatment of chronic diseases, beyond the traditional nutrients they contain. It should be pointed out that the term “functional” traditionally refers to the ability of a food ingredient, such as a soy protein product, to impart certain physiochemical properties to a food system. Thus, its meaning depends on the context. This may cause some confusion for certain readers.

Initially viewed as a passing fad, the concept of formulating foods for their ancillary health benefits is a trend that is quickly moving into the corporate mainstream. The market, estimated at several billion dollars, is global and growing fast. It is being further driven by the aging of the population, rising health care costs, and advancing food technology and human nutrition. There is an instant connection between functional foods and soy, because among the many plant and animal sources of functional foods, soy ranks the highest in terms of the number of phytochemicals it contains and the ability of its protein to lower cholesterol levels.

In line with this exciting development, this book, *Soybeans as Functional Foods and Ingredients*, has been developed. The key objective is to provide up-to-date information on soybean chemistry, health benefits, research, and product development so that readers can find answers to key questions: What are the nutrients and phytochemicals in soybeans? How can soybeans be utilized as food and as food ingredients so that general populations can reap the health benefits of soy? How can processing and breeding technology help expand soybean food utilization?

Chapter 1 gives a general overview of many chemical constituents of soybeans, categorized as nutrients and phytochemicals, with respect to their occurrence, chemistry, health benefits, and changes upon processing. Chapter 2 describes various edible soy products in the market. This is to inform readers and consumers about the variety available so that they can make informed choices and reap the health benefits of soy by consuming these products. Chapters 3 and 4 provide detailed coverage of two key soy phytochemicals, isoflavones and saponins, with respect to chemistry, analysis, potential health benefits, and commercial production. Chapters 5, 6, and 7 deal with three soy protein products: soy flour, concentrate, and isolate, respectively. These chapters emphasize the processing technology, properties, and food applications of these key soy product categories. Chapter 8 focuses on various barriers to soy protein applications in food systems from a practical point of view. Chapter 9

deals specifically with soy molasses, a by-product of soy concentrate processing that has much potential as a functional food or as a starting material. Chapter 10 provides coverage of products from extrusion-expelling of soybeans, an alternative process to solvent extraction. The next three chapters discuss three types of traditional soyfoods in detail: green vegetable soybeans, tempeh, and soy sauce, respectively. Production, processing steps, and potential as a functional food or food ingredient are covered for each of these traditional soyfoods. The last chapter, Chapter 14, provides a unique perspective on historical and current efforts to breed specialty soybeans for traditional and new soyfood uses in the United States, China, Japan, and Australia. It also provides a detailed list of publicly released soyfood cultivars available from these countries.

A few years ago, I wrote and edited my first soybean book, *Soybeans: Chemistry, Technology and Utilization* (Aspen Publishers, 1997, 1999). The current volume is an extension of that book since there is little overlap between the two. There are several unique features about this new volume. First, it can help readers to quickly develop an understanding of various nutrients and phytochemicals in soybeans, as well as various types of soyfoods in the current market. Second, it provides comprehensive coverage of each soy protein ingredient, a major way of using soy as food in the West, with respect to current processing technology and application strategies. Third it includes detailed treatment of two major soy nutraceuticals, isoflavones and saponins, as well as a thorough discussion of soy molasses, a common cost-effective starting material for development of nutraceutical products. Extensive patent review on commercial production of soy isoflavones is also included. Fourth, this volume also includes, in unprecedented length a unique discussion of historical and current undertakings to breed specialty soybeans for making traditional and modern soyfoods.

The current volume is written to serve as a timely and up-to-date reference for food product developers, food technologists, nutritionists, plant breeders, academic and governmental professionals, college graduates, and anyone who is interested in learning more about soybeans, soyfoods, soy protein ingredients, and soy nutraceuticals.

This book would have been impossible to complete without assistance from our chapter contributors. I would like to express my sincere appreciation to the 18 individual contributors who have expended so much of their time and energy outside their regular responsibilities in the preparation of their respective chapters. Their contributions denote a sincere dedication to their chosen profession and to the advancement of soybean chemistry and technology. I would also like to thank reviewers of each chapter manuscript for their valuable input and constructive suggestions. An alphabetical list of all reviewers is included in this book.

Special thanks are extended to Jean Wills, Executive Vice President of the American Oil Chemists' Society (AOCS), Mary Lane, retired director of AOCS Press, members of the Books and Special Publications Committee, and AOCS staff (particularly, Daryl Horrocks and Connie Winslow) for supporting and facilitating the book project, and to Ruth Kwon and Terri Gitler of Publication Services, Inc.

(Champaign, IL) for copyediting and producing the book. Their support and assistance, along with close co-operation from all the authors are critical elements toward successful execution of this project. Thanks are also expressed to the readers of my first book, *Soybeans: Chemistry, Technology and Utilization*, and to my professional colleagues, friends and family members, for their encouragement and support.

KeShun Liu, Ph.D.
June 2004

Contributing Authors

Thomas E. Carter, Jr., Ph.D., United States Department of Agriculture, Agricultural Research Service, Raleigh, NC, 27607, USA

Daniel Chajuss, Ph.D., Hayes General Technology Co. Ltd., Misgav Dov 19, Mobile post Emek Sorek 76867, Israel

Zhanglin Cui, Ph.D., North Carolina State University, Crop Science Department, 3127 Ligon St, Raleigh, NC, 27607, USA

Russ Egbert, Ph.D., Archer Daniels Midland Company, 4666 East Faries Parkway, Decatur, IL, 62526, USA

J. L. Kiers, Ph.D., Friesland Nutrition Research, Friesland Coberco Dairy Foods, P.O. Box 226, 8901 MA Leeuwarden, The Netherlands

A.T. James, Ph.D., CSIRO Division of Plant Industries, 120 Meiers Road, Indooroopilly 4068 Queensland, Australia

Lawrence A. Johnson, Ph.D., Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, 50011, USA

William Limpert, Cargill Inc., Research Department, P.O. Box 5699, Minneapolis, MN, 55440, USA

Jun Lin, Ph.D., Department of Nutrition, Food Science and Hospitality, South Dakota State University, Brookings, SD, 57006, USA

KeShun Liu, Ph.D., Department of Food Science, University of Missouri, Columbia, MO, 65211, USA

Rao S. Mentreddy, Department of Plant and Soil Science, Alabama A&M University, Normal, AL, 35762, USA

Shoji Miyazaki, Ph.D., National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba 305-8602, Japan

Ali I. Mohamed, Ph.D., Department of Biology, Virginia State University, Petersburg, VA, 23806, USA

Deland J. Myer, Ph.D., Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, 50011, USA

M.J.R. Nout, Ph.D., Laboratory of Food Microbiology, Wageningen University, 6700, EV Wageningen, The Netherlands

Leslie L. Skarra, Merlin Development, 181 Cheshire Lane, Suite 500, Plymouth, MN, 55441, USA

Chunyang Wang, Ph.D., Department of Nutrition and Food Science, South Dakota State University, Brookings, SD, 57006, USA

Tong Wang, Ph.D., Dept. of Food Science and Human Nutrition, Iowa State University, Ames, IA, 50011. USA

Richard F. Wilson, Ph.D., United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, 20705, USA

Reviewers

Sam K.C. Chang, Ph.D., Department of Cereal Science, North Dakota State University, Fargo, ND, 58105, USA

Russ Egbert, Ph.D., Archer Daniels Midland Company, Decatur, IL, 62526, USA

Junyi Gai, Professor, National Center of Soybean Improvement, Nanjing Agricultural University, Nanjing, Jinagsu, China

Xiaolin Huang, Ph.D., The Solae Company, St. Louis, MO, 63188, USA

Thomas Herald, Ph.D., Department of Animal Science and Industry, Kansas State University, Manhattan, KS, 66508, USA

Peter Golbitz, President, Soyatech Inc., Bar Harbor, ME, 04609, USA

Ingolf U. Gruen, Ph.D. Department of Food Science, University of Missouri, Columbia, MO, 65211, USA.

Mark Messina, Ph.D., Nutrition Matters, Inc., Port Townsend, WA, 98368, USA.

S. Shanmugasundaram, Ph.D., Asian Vegetable Research and Development Center, Shanhua, Taiwan

Chunyang Wang, Ph.D., Department of Nutrition and Food Science, South Dakota State University, Brookings, SD, 57006, USA

Richard F. Wilson, Ph.D., United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, 20705, USA

Contents

Preface

Contributing Authors

Reviewers

- Chapter 1 **Soybeans as a Powerhouse of Nutrients and Phytochemicals**
KeShun Liu
- Chapter 2 **Edible Soybean Products in the Current Market**
KeShun Liu
- Chapter 3 **Soy Isoflavones: Chemistry, Processing Effects, Health Benefits, and Commercial Production**
KeShun Liu
- Chapter 4 **Soybean Saponins: Chemistry, Analysis, and Potential Health Effects**
Jun Lin and Chunyang Wang
- Chapter 5 **Soy Flour: Varieties, Processing, Properties, and Applications**
KeShun Liu and William F. Limpert
- Chapter 6 **Soy Protein Concentrate: Technology, Properties, and Applications**
Daniel Chajuss
- Chapter 7 **Isolated Soy Protein: Technology, Properties, and Applications**
William Russell Egbert
- Chapter 8 **Barriers to Soy Protein Applications in Food Products**
Leslie Skarra
- Chapter 9 **Value-Added Products from Extruding-Expelling of Soybeans**
Tong Wang, Lawrence A. Johnson, and Deland J. Myers
- Chapter 10 **Soy Molasses: Processing and Utilization as a Functional Food**
Daniel Chajuss
- Chapter 11 **Vegetable Soybeans as a Functional Food**
Ali Mohamed and Rao S. Mentreddy

Chapter 12 **Tempeh as a Functional Food**

M.J.R. Nout and J.L. Kiers

Chapter 13 **Soy Sauce as Natural Seasoning**

KeShun Liu

Chapter 14 **Breeding Specialty Soybeans for Traditional
and New Soyfoods**

*Zhanglin Cui, A.T. James, Shoji Miyazaki, Richard F. Wilson,
and Thomas E. Carter, Jr.*

Chapter 1

Soybeans as a Powerhouse of Nutrients and Phytochemicals

KeShun Liu

University of Missouri, Columbia, MO 65211

Soybean belongs to the family Leguminosae. The cultivated form, *Glycine max* (L.) Merrill, grows annually. The plant is bushy with height ranging from 0.50 to 1.25 m. Soybean seeds are spherical to long oval. Most of the seeds are yellow, but some are green, dark brown, purplish black, or black.

Historical and geographical evidence indicates that soybean originated in northern China, and its cultivation in the region started as early as the New Stone Age, some 5,000 years ago (1). Soybean (then known as *shu*, now as *da dou* or *huang dou* in Chinese) was repeatedly mentioned in later records, and was considered one of the five sacred grains, along with rice, wheat, barley, and millet. During the course of soybean cultivation, the Chinese had gradually transformed soybean into various types of tasty and nutritious soyfoods, including tofu, soymilk, soy sprouts, soy paste, and soy sauce. Along with soybean cultivation, methods of soyfood preparation were gradually introduced to Japan, Korea, and some other Far East countries about 1,100 years ago. Peoples in these countries not only accepted the Chinese way of preparing soyfoods, but also modified the methods and even created their own types of soyfoods. Soybean was first introduced to Europe and North America in the 18th century. However, large-scale official introduction into the United States did not occur until the early 1900s. Thousands of new varieties were brought in, mostly from China, during this period. Until 1954, China led the world in soybean production. Since then the United States has become the world leader.

Since the 1950s, the soybean has emerged as one of the most important agricultural commodities in the world, with a steady increase in annual production (Fig. 1.1). Currently, global production is estimated at 180 million metric tons. Major producers include the United States, Brazil, Argentina, China, and India. In any fiscal year, U.S. farmers produce about half of the total world soybean harvest, with more than one-third of the U.S. production exported (2).

As a crop, soybeans have several favorable features. First, soybean has an ability to fix nitrogen, which makes it a good rotational crop. Second, soybeans are adaptable to a wide range of soils and climates. Third, soybean has the remarkable ability to produce more edible protein per acre of land than any other known crop. On average, dry soybean contains roughly 40% protein, 20% oil, 35% carbohydrate, and 5% ash. Thus, soybean has the highest protein content among cereal and other legume species, and the second-highest oil content among all food legumes. Fourth,

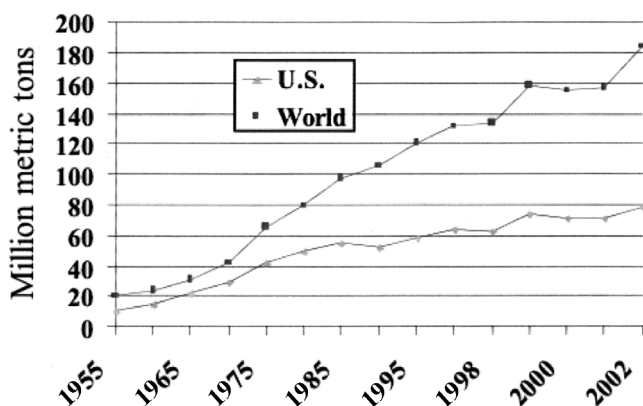


Figure 1.1. U.S. and world annual production of soybeans since 1955 (2).

soybean has versatile end uses. Broadly speaking, it can be used as human food, animal feed, and industrial material. Currently, the majority of annual soybean production is crushed into oil, for use in foods and food processing, and defatted meal, for use as animal feed. Only a small fraction is processed into whole-bean foods for direct human consumption (2).

For many years soybeans have been recognized as a powerhouse of nutrients. The protein and oil components in soybeans are high in quality as well as in quantity. Soy oil contains a high proportion of unsaturated fatty acids, including oleic, linoleic, and linolenic acids. The last two are essential fatty acids for humans. Soy protein contains all the essential amino acids, most of which are present in amounts that closely match those required by humans or animals.

Current technologies have revolutionized soybean research. Successful application of biotechnology has led to the development of new soybean varieties with herbicide tolerance, pest resistance, and/or altered chemical composition. Medical research continues to elucidate the roles of soy in preventing and treating such chronic diseases as heart disease, cancer, and bone diseases. Technology has also provided new ways of producing nutraceuticals and industrial materials from soybean (3–6). Although biotechnology, crop and production improvement, and animal feed uses have driven soybean production to an all-time high, it is the recent medical discoveries regarding the health benefits of soy that have led to a worldwide interest in using soy in food and nutraceutical products. Thousands of studies—in vivo and in vitro, with animals and human subjects—have shown that soybeans and soy components have many health-promoting effects, including hypocholesterolemic, anticancer, and antioxidant. Regular consumption of soy can help reduce heart disease, prevent breast and prostate cancers, improve bone health and memory, and alleviate menopausal symptoms in some women. Many types of biologically active

components have been shown to be partially responsible for these effects. Although isoflavones have been recognized as key components responsible for the health-promoting effects, many other bioactive components of soybeans are also of interest, such as lecithin, saponins, lectins, oligosaccharides, and trypsin inhibitors. Most of these components are traditionally known as antinutritional factors, but now are known as phytochemicals. These components, although present in minor quantities as compared with protein and oil, can exert some unique health benefits for animals and humans. In this regard, soybean is now known as a powerhouse of phytochemicals as well. Table 1.1 lists general contents of nutrients as well as some phytochemicals in soybeans on a dry matter basis. Additional information can be found on the U.S. Department of Agriculture website (26).

TABLE 1.1

General Concentrations of Nutrients and Phytochemicals in Soybeans (Dry Matter Basis)

Component	Unit	Range	Typical	References
Protein	%	30–50	40	Orf 1988 (7); Liu, Orthoefer, and Brown 1995 (8)
Amino acid composition	g/100 g seed			Han, Parsons, and Hymowitz 1991 (9)
<i>Non-essential</i>				
Alanine		1.49–1.87	1.69	
Arginine		2.45–3.49	2.90	
Aspartic acid		3.87–4.98	4.48	
Glutamic acid		6.10–8.72	7.26	
Glycine		1.88–2.02	1.69	
Cysteine		0.56–0.66	0.60	
Proline		1.88–2.61	2.02	
Serine		1.81–2.32	2.07	
<i>Essential</i>				
Histidine		0.89–1.08	1.04	
Isoleucine		1.46–2.12	1.76	
Leucine		2.71–3.20	3.03	
Lysine		2.35–2.86	2.58	
Methionine		0.49–0.66	0.54	
Phenylalanine		1.70–2.08	1.95	
Threonine		1.33–1.79	1.58	
Tryptophan		0.47–0.54	0.49	
Tyrosine		1.12–1.62	1.43	
Valine		1.52–2.24	1.83	
Oil		12–30	20	Orf 1988 (7); Liu, Orthoefer, and Brown 1995 (8)
Fatty acid composition	% relative to total oil			Hammond and Glatz 1988 (10), Liu 1999 (11), Fehr and Curtiss 2004 (12).
Palmitic acid		4–23	11	
Stearic acid		3–30	4	

(Continued)

TABLE 1.1*(Cont.)*

Component	Unit	Range	Typical	References
Fatty acid composition	% relative to total oil			Hammond and Glatz 1988 (10), Liu 1999 (11), Fehr and Curtiss 2004 (12).
Oleic acid		25–86	25	
Linoleic acid		25–60	53	
Linolenic acid		1–15	7	
Carbohydrates	%	26–38	34	Orf 1988 (7); Liu, Orthoefer, and Brown 1995 (8)
Sucrose		2.5–8.2	5.5	Hymowitz <i>et al.</i> 1972 (13)
Raffinose		0.1–0.9	0.9	Hymowitz <i>et al.</i> 1972 (13)
Stachyose		1.4–4.1	3.5	Hymowitz <i>et al.</i> 1972 (13)
Ash	%	4.61–5.94	5.0	Taylor <i>et al.</i> 1999 (14)
Vitamins				
Thiamine	µg/g	6.26–6.85		Fernando and Murphy 1993 (15)
Riboflavin	µg/g	0.92–1.19		Fernando and Murphy 1993 (15)
Vitamin E	µg/g			Guzman and Murphy 1986 (16)
α-tocopherol		10.9–28.4		
γ-tocopherol		150–190		
δ-tocopherol		24.6–72.5		
Isoflavones	%	0.1–0.4	2.5	Coward <i>et al.</i> 1993 (17), Wang and Murphy 1994 (18)
Saponins	%	0.1–0.3		Arditi, Meredith, and Flowerman 2000 (19)
Phytate	%	1.0–1.5	1.1	Lolas, Palamidis, and Markakis 1976 (20)
Phytosterols	mg/g	0.3–0.6		Rao and Janezic 1992 (21)
Trypsin inhibitors	mg/g	16.7–27.2	22.3	Liener 1994 (22), Anderson and Wolf 1995 (23)
Lectin	HU*/mg protein	1.2–6.0	3.0	Padgett <i>et al.</i> 1996 (24)
Lunasin	% defatted flour	0.33–0.95	0.65	De Mejia, Wang, <i>et al.</i> 2004 (25)

*HU = Hemagglutinin unit.

In this chapter, macro- and micronutrients and biologically active components are discussed with respect to their natural occurrence, nutritional value, and health benefits. Detailed coverage of these components is beyond the scope of this book and can be found elsewhere (11,27–32).

Soy Proteins

The main nutritional component present in soybeans is protein. Based on biological function in plants, seed proteins are of two types: metabolic proteins and storage proteins. Storage proteins, together with reserves of oils, are synthesized during soybean seed development. The majority of soybean protein is storage protein. The two

main types of storage proteins are glycinin and beta-conglycinin, also known as 11S and 7S protein, respectively (33,34).

Soy protein is a major component of the diet of food-producing animals and is increasingly important in the human diet. Soy protein is considered deficient in sulfur-containing amino acids, but it does contain all 11 of the essential amino acids required for human or animal nutrition, namely isoleucine, leucine, lysine, methionine, cyst(e)ine, phenylalanine, tyrosine, threonine, tryptophan, valine, and histidine (35). Adverse nutritional and other effects following consumption of raw soybean meal have been attributed to the presence of endogenous inhibitors of digestive enzymes and lectins and to poor digestibility. To improve the nutritional quality of soy proteins, heat inactivation of these naturally occurring biologically active compounds is needed. A general review of the nutritional and health benefits of soy protein can be found in the literature (31).

When the protein quality is expressed as the *protein digestibility-corrected amino acid score* (PDCAAS):

$$\text{PDCAAS} = \frac{\text{amino acid pattern of a protein}}{\text{amino acid requirements for an organism}} \times \text{digestibility of the protein}$$

instead of as the *protein efficiency ratio* (PER), soy protein, when in a purified form, is equivalent in quality to animal proteins (36–38). Soy protein has a PDCAAS very close to 1, the highest rating possible. PDCAAS is a measure of how limiting the limiting amino acid is in a protein after an adjustment for digestibility, whereas PER is based on a rat feeding assay, which tends to underestimate the quality of soy protein.

In addition to being of high quantity and high quality, soy protein is hypocholesterolemic. Because of soy's effectiveness in lowering total cholesterol as well as low-density lipoprotein (LDL) cholesterol (39), formal recognition of the cholesterol-lowering properties of soy protein came in 1999 when the U.S. Food and Drug Administration (FDA) approved a health claim for the cholesterol-lowering effects of soy protein. The following claim may now be used on qualified soy products: "Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease" (40). Although the FDA set 25 g per day as the target intake goal for cholesterol reduction, some data suggest that fewer than 25 g per day are also effective for cholesterol reduction (32). The cholesterol-lowering effects of soy protein are less than that of cholesterol-lowering drugs such as statins—the average decrease in LDL cholesterol in response to soy protein is about 6% compared to placebo—but every 1% decrease in LDL can lower coronary heart disease risk as much as 4%. Certainly, soy protein can be a very important part of an overall heart-healthy diet (41). While the FDA-approved health claim is based on soy protein content, a number of other physiologically active components may contribute to the inherent cholesterol-lowering effect. These include amino acids, isoflavones, saponins, phytic acid, trypsin inhibitors, fiber, and globulins (storage proteins in soy). A statement for healthcare professionals from the Nutrition Committee of the American Heart Association on soy protein and cardiovascular disease is available (42).

Recently, Japanese researchers carried out a study to investigate the effects of soybean beta-conglycinin (7S-globulin) and glycinin (11S-globulin) on serum lipid levels and metabolism in the livers of normal and genetically obese mice. Male normal and obese mice were fed high-fat diets for two weeks, followed by a two-week restricted diet (2 g diet/mouse/day) containing 20% casein, soybean beta-conglycinin, or soybean glycinin, and then sacrificed immediately. Results indicate that serum triglyceride, glucose, and insulin levels of beta-conglycinin-fed mice were lower than in casein- and glycinin-fed mice of both strains, suggesting that soy beta-conglycinin could be a potentially useful dietary protein source for the prevention of hypertriglyceridemia, hyperinsulinemia, and hyperglycemia, which are recognized as risk factors for atherosclerosis (43).

Another benefit of soy protein is the favorable effect on renal function compared to animal protein (44). Although long-term studies are needed, this attribute of soy protein may be very important because the increasing incidence of diabetes will lead to a greater incidence of renal problems. In people with mild renal insufficiency, high protein intake adversely affects renal function (45). Therefore, substituting soy protein for some of the animal protein in the diet represents an alternative to restricting total protein intake.

Soy protein has been shown to decrease urinary calcium excretion when substituted for animal protein, such as meat and milk protein (46,47). Decreasing dietary calcium requirements may help to reduce the risk of osteoporosis because relatively few women meet dietary calcium requirements.

Soybean Oil

During seed development, soybeans store their lipids in organelles known as oil bodies, mainly in the form of triglycerides. During processing, components extracted from soybeans by organic solvents such as hexane are classified as crude oil. Major components of crude oil are triglycerides (or triacylglycerols). Minor components include phospholipids, unsaponifiable material, free fatty acids, and trace metals. Unsaponifiable material consists of tocopherols, phytosterols, and hydrocarbons. The concentrations of these minor compounds are reduced after typical processes of oil refinement. Thus, refined soybean oil contains more than 99% triglycerides. Triglycerides are neutral lipids, each consisting of three fatty acids and one glycerol, which links the three acids. There is a large genetic variation in fatty acid composition of soybean oil, mainly resulting from plant breeding. The range of fatty acid composition among soybean germplasm has been reported to be palmitic acid (C16:0), 4–23%; stearic acid (C18:0), 3–30%; oleic acid (C18:1), 25–86%; linoleic acid (C18:2), 25–60%; and linolenic acid (C18:3), 1–15% (10,48). However, typical soybean oil has a fatty acid composition of C16:0, 11%; C18:0, 4%; C18:1, 24%; C18:2, 53%; and C18:3, 7%.

The soybean is one of the few good plant sources of two essential fatty acids, linoleic acid and linolenic acid. These fatty acids are considered essential because mammals, including humans, cannot synthesize them and they therefore must be ob-

tained from the diet. Linolenic acid is also an omega-3 fatty acid. Chapkin (49) reported that many populations have diets low in the omega-3 fatty acids.

Increasing evidence indicates that types and levels of fats and oils consumed have a significant influence on the well-being of the general population. Dietary lipids have been found to play a significant role in the pathogenesis of cardiovascular diseases, cancer, and other disorders. This role depends on the length of the carbon skeleton, and on the number and the geometry of the double bonds. In general, saturated fatty acids raise total cholesterol levels whereas mono- and polyunsaturated fatty acids exhibit a lowering effect. The risk of coronary heart disease (CHD) rises as serum total and LDL cholesterol concentrations increase, and declines with increasing levels of high-density lipoprotein (HDL) cholesterol (50,51). Since natural soy oil (without hydrogenation) is cholesterol-free, low in saturated fatty acids (about 15% total), and high in unsaturated fatty acids (about 85% total), it is considered a healthy oil.

Carbohydrates and Oligosaccharides

On average, dry soybeans contain about 35% carbohydrates. Among the soluble carbohydrates, raffinose and stachyose garner more attention, mainly because their presence has been linked to flatulence and abdominal discomfort associated with human consumption of soybeans and soy products. In soybeans, the content of raffinose ranges from 0.1% to 0.9% on a dry matter basis, and stachyose from 1.4% to 4.1% (13). These oligosaccharides are nonreducing sugars, containing fructose, glucose, and galactose as three or four units, linked by β -fructosidic and α -galactosidic linkages (Fig. 1.2). Humans are not endowed with the enzyme (α -galactosidase) necessary for hydrolyzing the α -galactosidic linkage present in these oligosaccharides. Humans cannot digest the oligosaccharides in the duodenal and small intestinal mucosa. The intact sugars go directly into the lower intestine, where they are metabolized by microorganisms that contain the necessary enzyme. This results in production of such gases as carbon dioxide, hydrogen, nitrogen, and methane, depending on the individual diet and microflora spectrum. Consequently, the host experiences flatulence and other undesirable side effects (22,52).

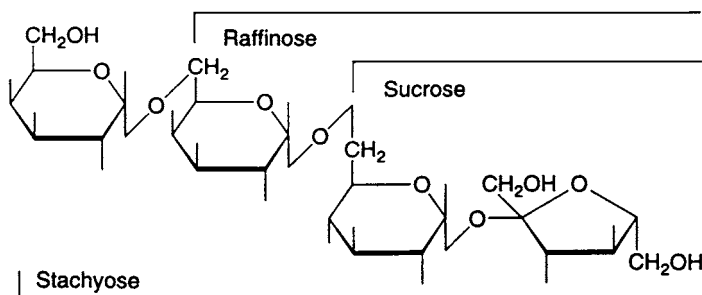


Figure 1.2. Molecular structure of oligosaccharides in soybeans.

The insoluble carbohydrates in soybeans include cellulose, hemicellulose, pectin, and a trace amount of starch. They are structural components found mainly in cell walls. The seed coat represents approximately 8% of the whole soybean by dry weight, but contains about 86% complex carbohydrates. Thus, the seed coat contributes a noticeable amount of insoluble carbohydrates to the whole bean.

The majority of carbohydrates of soybeans (oligosaccharides and complex polysaccharides) fall into a general category known as dietary fiber. According to a general definition, dietary fiber consists of the endogenous components of plant material in the diet, which are resistant to digestion by humans. The effect of dietary fiber in human diets on nutritional response has received increasing attention during the last few decades. Medical research has indicated a clear relationship between several common diseases in affluent societies and lack of fiber in the diet (53). Certain physiological responses have been associated with the consumption of dietary fiber. These responses include an increase in fecal bulk, a reduction in plasma cholesterol, a blunting of the postprandial increase in plasma glucose, and a decrease of nutrient bioavailability (54,55).

The health benefits of dietary fiber are particularly relevant to soy oligosaccharides. Although their presence is generally considered undesirable with respect to their flatus activity, new studies have shown that dietary oligosaccharides can exert many positive benefits, including (a) increasing the population of indigenous bifidobacteria in the colon, which, by their antagonistic effect, suppress the activity of putrefactive bacteria; (b) reducing toxic metabolites and detrimental enzymes; (c) preventing pathogenic and autogenous diarrhea by the same mechanisms as those by which they reduce detrimental bacteria; (d) preventing constipation due to production of high levels of short-chain fatty acids by bifidobacteria; (e) protecting liver function by reducing the production of toxic metabolites; (f) reducing blood pressure; (g) having anticancer effects; and (h) producing nutrients such as vitamins, also due to increased activity of bifidobacteria (56,57). In Japan, oligosaccharides have been developed into one of the most popular functional food components (57).

Vitamins and Minerals

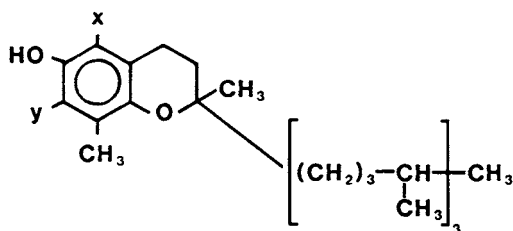
Soybeans contain both water-soluble and oil-soluble vitamins. The water-soluble vitamins present in soybeans mainly include thiamin, riboflavin, niacin, pantothenic acid, and folic acid. These are not substantially lost during oil extraction and subsequent toasting of flakes. Fernando and Murphy (15) reported consistent recoveries of 84% for thiamin and 95% for riboflavin in the whole soy flour made from three soybean varieties. The contents in these samples ranged from 6.26 to 6.80 $\mu\text{g/g}$ and from 0.92 to 1.19 $\mu\text{g/g}$ for thiamin and riboflavin, respectively. However, the researchers also reported that during processing of soybeans involving water, such as tofu making, losses of these vitamins were remarkable. The ranges of retention for both thiamin and riboflavin in tofu were found to be 7.6–15.7% and 11.7–21.1% respectively. The amount of ascorbic acid (vitamin C) is essentially negligible in ma-

ture soybeans, although it is present in measurable amounts in both immature and germinated seeds (58).

The oil-soluble vitamins present in soybeans are vitamins A and E, with essentially no vitamins D and K. Vitamin A exists mainly as the provitamin β -carotene. Like ascorbic acid, its content is negligible in mature seeds but measurable in immature and germinated seeds (58). Vitamin E is also known as tocopherol and has four isomers, α -, β -, γ - and δ -tocopherols (Fig. 1.3). According to Guzman and Murphy (16), the tocopherol content varies significantly from one soybean variety to another. The amounts of α -, γ -, and δ -tocopherols in soybeans ranges from 10.9 to 28.4, 150 to 191, and 24.6 to 72.5 $\mu\text{g/g}$ (on a dry matter basis), respectively. Processing of soybeans into tofu results in 30–47% loss of vitamin E, but the tofu is a greater source of tocopherols than the whole beans on a dry basis. Pryde (59) reported that crude soy oil contains 9–12 mg/g of α -tocopherol, 74–102 mg/g of γ -tocopherol, and 24–30 mg/g of δ -tocopherol. The amount of β -tocopherol in soybeans is insignificant, being less than 3% of the total.

Vitamin E is retained in the oil during solvent extraction of soybeans. In fact, vitamin E is considered an important constituent of soy oil partly because of its nutritional and antioxidant properties. All tocopherol isomers tend to decrease during oil refinement, with γ -tocopherol losing the most. The isomers are lost mainly in the deodorization step.

Dry soybeans have an ash content of about 5%. Among the major mineral components in soybeans, potassium is found to be in the highest concentration, followed by phosphorus, magnesium, sulfur, calcium, chloride, and sodium. The contents of these minerals range from 0.2 to 2.1% on average. The minor minerals present in soybeans and soy products include silicon, iron, zinc, manganese, copper,



α-tocopherol	$x=y=\text{CH}_3$
β-tocopherol	$x=\text{CH}_3$, $y=\text{H}$
γ-tocopherol	$x=\text{H}$, $y=\text{CH}_3$
δ-tocopherol	$x=y=\text{H}$

Figure 1.3. Molecular structure of vitamin E (tocopherols) in soybeans.

molybdenum, fluorine, chromium, selenium, cobalt, cadmium, lead, arsenic, mercury, and iodine. The contents of these minor minerals range from 0.01 to 140 ppm (11,60). During processing, the majority of mineral constituents follow the protein or meal portion of soybeans rather than the oil.

Lecithin

Lecithin is a main by-product of soy oil refining processes and constitutes 0.5–1.5% of soybean seed, or 1–3% of crude soybean oil. The total phospholipids in soybeans are about 35% phosphatidyl choline, about 25% phosphatidyl ethanolamine, about 15% phosphatidyl inositol, and 5–10% phosphatidic acid; the rest is a composite of all the minor phospholipid compounds. The parent compound is phosphatidic acid, which is not present in the free form in active cells except as an intermediate in the biosynthesis of other phosphoglycerides. Others are esters of phosphatidic acid (Fig. 1.4).

Phospholipids are polar lipids. Their removal from crude oil is carried out by centrifugation following hydration at an elevated temperature, the process commonly known as degumming. Phospholipids are good emulsifying agents, soluble in alcohol and insoluble in acetone. In living tissues, they are the major components of cell membranes. The common name of phosphatidyl choline is lecithin. However, in broad usage, the term “lecithin” generally refers to the entire phospholipid fraction separated from soybean crude oil by degumming.

Lecithin is an important source of choline, which is essential for the signaling functions and structural integrity of cells and also provides a source of the methyl group necessary for normal metabolism (61). The therapeutic benefits of lecithin include lowering of cardiovascular disease risk, prevention of abnormal fetal development, reduction of some forms of male infertility, promotion of healthy liver function, improvement in memory and cognition, and prevention or reduction of adverse reactions to various drugs. Lecithin appears to reduce plasma homocysteine levels. Increased risk of coronary heart disease and stroke has been associated with high plasma homocysteine levels (62). Homocysteine is formed *via* demethylation

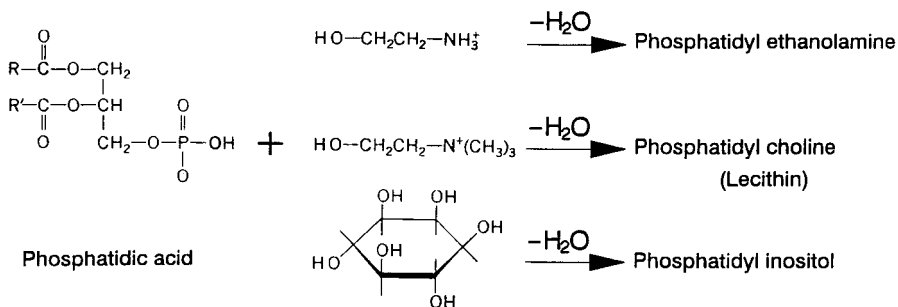


Figure 1.4. Molecular structure of phospholipids in soybeans.

of methionine. Plasma levels of homocysteine may increase due to deficiencies in vitamins B₆, B₁₂, and folate, but choline deficiency may serve as a risk factor for hyperhomocysteinemia as well. Ghoshal and Farber (63) reported that choline deficiency may result in fatty infiltration of the liver. Kneuchel (64) found that men who consumed 1.35 g of phosphatidyl choline per day had a significantly improved liver function as compared to the placebo group. Thus, lecithin has hepatoprotectant effect. Furthermore, lecithin is known to provide choline to neurons in the central nervous system. Acetylcholine has long been recognized as a neurotransmitter in the mammalian brain. Its effects include control of movement, sleep, and memory. While lecithin has little therapeutic value for Alzheimer's dementia, it might be able to improve memory in non-demented individuals (65).

Isoflavones

Although flavonoids are found in various plant families in different tissues, isoflavones are present in just a few botanical families. The soybean is unique in that it contains the highest amount of isoflavones, being in the range of 0.1–0.4% dry weight (17,66–69)

The isoflavones in soybeans and soy products are of three basic types: daidzein, genistein, and glycitein. Each of these three isomers, known as aglucones or free forms, can also exist in three conjugate forms: glucoside, acetylglucoside, and malonylglucoside. Therefore, in total, there are 12 isomers of isoflavones in soybeans (11,18). The major isoflavones in soybean are daidzin and genistin, the β -glucoside forms of daidzein and genistein, respectively. Comprehensive analysis of isoflavone contents in numerous soy food products indicates that most products contain 0.1–0.3% of total isoflavone (17,68).

Among all the health-promoting components of soy, isoflavones are thought to be most responsible for many of the hypothesized health benefits of soyfoods, and thus have gained most attention in scientific community. Approximately 600 scientific papers are published on isoflavones each year. The potential health benefits include prevention and treatment of cardiovascular disease, cancer, osteoporosis, and premenstrual and postmenopausal symptoms, among others (32,69). Chapter 3 provides detailed coverage of soy isoflavones.

Soy Saponins

Saponins are composed of sugars bound to alkaloid, steroid, or triterpene compounds and have detergent surfactant properties. The aglycone portions of saponins are known as genin or sapogenin. Soy proteins contain 0.1–0.3% saponins, at least five of which have been isolated (19). Many studies have shown that saponins have blood cholesterol-lowering properties rendered by their binding of cholesterol. The bound cholesterol is then passed into the colon and excreted. Saponins have also been shown to reduce the risk of cancer and heart disease. The binding of bile acids by saponins removes cholesterol metabolites from the colon and hence reduces the risk of colon cancer. In addition, saponins inhibit cancer cell proliferation by binding to them (70). Chapter 4 provides detailed coverage of saponins.

Phytosterols

Phytosterols are lipid-like compounds found in plants. Soybeans, rapeseeds, and coniferous trees are the three major commercial sources of phytosterols. Campesterol, β -sitosterol, and stigmasterol are the three major phytosterols in soybeans and most other plants (Fig. 1.5). These particular sterols are 4-desmethyl sterols that share an identical ring structure with cholesterol, but differ only in respective side chains. The presence of a side-chain substituent of a methyl (campesterol) or an ethyl (sitosterol) group distinguishes different sterols. Moreover, the additional double bond at position 22 is unique for stigmasterol. Hydrogenation of sterols results in formation of stanols. Plant stanols are a less abundant class of sterols found in oilseed. About 2% of total phytosterols in soybeans are stanols. The structure of sterols and stanols resembles that of cholesterol found in animals. Their essential role in plants is to stabilize cell membranes, similar to the role of cholesterol in animals (71,72).

The total phytosterol content of soybeans is estimated at 0.3–0.6mg/g. Soybean sterols and other sterols derived from oilseeds are obtained during oil processing as by-products of vitamin E manufacturing (21,29).

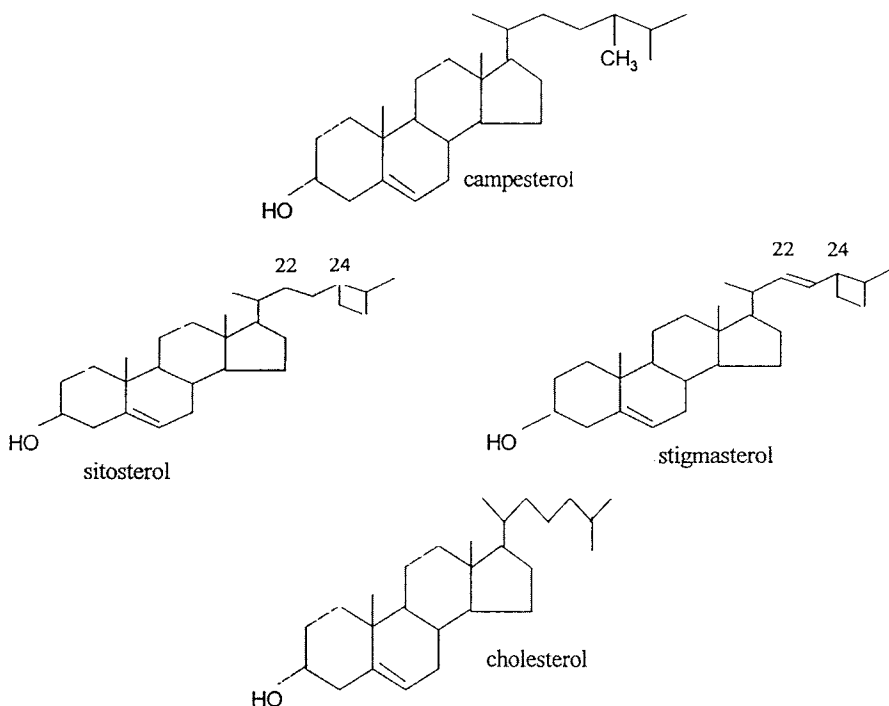


Figure 1.5. Molecular structure of phytosterols in soybeans as compared with that of cholesterol.

Although phytosterols are structurally related to cholesterol, they have been clinically proven to reduce blood cholesterol in humans. In fact, phytosterols represent one of the most intensely studied nutraceuticals in the area of cardiovascular diseases. For over 50 years, numerous studies have reported a cholesterol-lowering property associated with phytosterols and stanols, which may as a consequence contribute to a reduced risk of coronary heart disease. Some studies have demonstrated that the ingestion of 3–6 g of sitosterol per day leads to a decrease in total serum cholesterol of 7–9%. Most of the published data show that a daily intake of 2–3 g of phytosterols lowers LDL cholesterol levels by 10–15%. This means that consumption of 2 g/day may reduce the risk of heart disease by about 25%. There is a dose-response relationship between consumption of sterols and cholesterol reduction (71). The role of dietary phytosterols in colon carcinogenesis has also been reported (21).

Plant sterols and stanols are consumed at approximately 100–300 mg/day and 20–50 mg/day, respectively, as part of a typical Western diet. Thus, fortification of conventional foods with plant sterols can significantly increase the daily intake of sterols and help reduce cholesterol levels. In the United States, the FDA has approved uses of stanols and sterol esters in margarine products, such as Benecol and Take Control, and classified them as GRAS (generally recognized as safe). Newer plant sterols, mainly from soybeans, are also being approved (73). More information on the subject can be found in the literature (71,72).

Phytate

Phytate is the calcium-magnesium-potassium salt of inositol hexaphosphoric acid, commonly known as phytic acid (Fig. 1.6). Phytic acid is also referred to as phytin in some literature. In many cereals and oilseeds, phytate is known to be located in

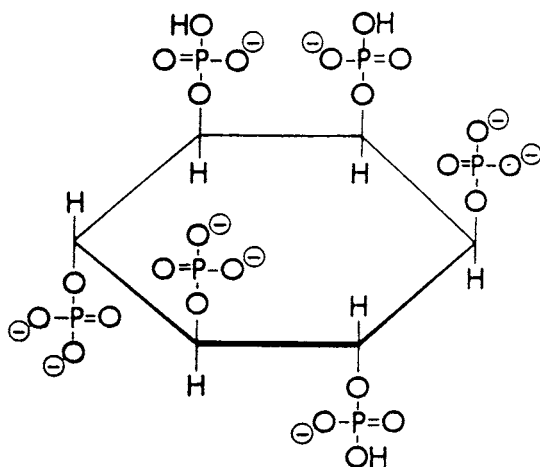


Figure 1.6. Molecular structure of phytic acid.

the protein bodies, mainly within their globoid inclusions (74). As in most seeds, phytate is the principal source of phosphorus in soybeans (22,75). The phytate content ranged from 1.00 to 1.47% on a dry matter basis and this value represented 51.4–57.1% of the total phosphorus in seeds (20). However, the actual content depends not only on variety, but also on growing conditions and assay methodology. The phytate content in several commercial soy protein products was also reported, with soy meal having a level of 1.42%, and flakes and isolates having a level of 1.52% (75).

Our interest in phytate arises mainly from its effect on mineral bioavailability and protein solubility when present in animal feed or human food. There is an abundance of literature that supports the theory that the requirement for certain metals in experimental animals is increased when soybeans are used as a source of protein in their diet (76–78). The effect has been attributed to the ability of phytic acid to chelate with di- and trivalent metal ions, such as Ca^{2+} , Mg^{2+} , Zn^{2+} , and Fe^{3+} , to form poorly soluble compounds that are not readily absorbed from the intestine. This conclusion is based on not only animal studies (76) but also human experiments (77) and in vitro studies (78).

Phytate is also capable of forming complexes with negatively charged protein molecules at alkaline pH through calcium- and magnesium-binding mechanisms, and with positively charged protein molecules at pH values below their isoelectric point by charge neutralization. As a consequence of this nonselective binding to proteins, phytate has been shown not only to inhibit the action of a number of enzymes important in digestion (79) but also to affect the isoelectric point, solubility, and functionality of soy proteins (80).

Phytic acid shows a remarkable antioxidant function by chelating pro-oxidant divalent metal ions such as those of iron and copper. Both in vivo and in vitro studies have demonstrated the striking anticancer effect of phytic acid (81).

Trypsin Inhibitors

Protease inhibitors are substances that, when added to a mixture of a protease (such as trypsin or chymotrypsin) and a substrate, bind to the enzyme and produce a decrease in the rate of substrate cleavage. Protease inhibitors of a protein nature are ubiquitous. Two types of protein proteinase inhibitors have been isolated from soybeans: Kunitz trypsin inhibitor and Bowman-Birk (BB) inhibitor. The Kunitz inhibitor has a MW between 20 and 25 kD, with a specificity directed primarily toward trypsin. The soybean BB inhibitor has a MW of 8 kD and is capable of inhibiting both trypsin and chymotrypsin at independent reactive sites.

Trypsin inhibitors are commonly assayed based on an enzymatic method using a synthetic substrate (82). Trypsin inhibitors are readily destroyed by heat treatment. Most processed soy products have a reduced enzymatic activity. Liener (22) reported 3.2–7.9 mg/g in soy flour, 6.3–13.7 mg/g in soy concentrate, and 4.4–11.0 mg/g in soy isolate. Compared with 52.1 mg/g in raw soy flour, this was a 75–95% reduction.

The significance of soybean trypsin inhibitors lies in their nutritional implications for both human and animals. Early studies found that soybean meal had to be heated in order to support the growth of rats. An assumption is that trypsin inhibitors present in soybeans are responsible for growth depression by reducing protein digestibility. Later studies showed that trypsin inhibitors themselves could cause hypertrophy of the pancreas in chicks. Since the pancreas is responsible for the production of most enzymes required for the digestion of food, dietary components that affect pancreatic function could markedly influence the availability of nutrients from the diet (22,83).

Much controversy has arisen in recent years regarding physiological roles of protease inhibitors as medical research demonstrates that protease inhibitors have the ability to serve as cancer-chemopreventive agents both *in vitro* and *in vivo*. At least one inhibitor in soybeans, BB inhibitor, has been shown to have clear anticarcinogenic activity in both *in vitro* and *in vivo* carcinogenesis assay systems (84,85). Unlike most of the other potential classes of cancer-chemopreventive agents that have been studied, protease inhibitors have the ability to affect the carcinogenic process in an irreversible manner and to affect many different kinds of carcinogenesis. Protease inhibitors are effective at extremely low levels, unlike most other agents. Therefore, even though the mechanism of action of protease inhibitors in the prevention of cancer is not yet elucidated, it is clear that the protease inhibitors are powerful anticarcinogenic agents (86).

Kennedy and Szuhaj (87) reported a method for making a Bowman-Birk inhibitor concentration for treatment of premalignant tissues. The method uses soy molasses as a starting material. The method involves dilution of soy molasses with water to 15–25% solids, centrifugation, and ultrafiltration to produce a crude BB inhibitor concentrate, which may be further purified by another ultrafiltration and precipitation with acetone.

Lectins

Lectins, also known as hemagglutinins, are proteins in nature and possess a remarkable ability to agglutinate erythrocytes and other types of cells. They are found predominantly in plant seeds, particularly those of the legumes, but they are also present in other parts of plants such as roots, leaves, and bark (88). Lectins are characterized by a relative high content of 4-hydroxyproline. The ability to agglutinate cells results from their ability to bind specifically to saccharides on the surface (membranes) of cells and act as bridges between cells.

Seed lectins are primarily localized in the protein bodies of the cotyledon cells. Soy lectin sedimentates with the 7S fraction during ultracentrifugation, and has a MW of approximately 120 kD and comprises four identical subunits, each with a MW of 30 kD. In addition to reacting with carbohydrates, the soybean hemagglutinin is a glycoprotein containing five glucosamine and 37 mannose residues per mole (89).

There are genetic variants for soy lectin levels. De Mejia and others (90) measured 144 selected and diverse soybean accessions from the USDA soybean germplasm collection grown under different environmental conditions using both ELISA and gel electrophoresis. They found that lectin concentration ranged from 1.1 to 14.5 mg/g of extracted protein. The highest concentration was found in exotic accessions. Like the trypsin inhibitors, soy lectin is readily destroyed by moist heat treatment. Soy lectin's inactivation closely parallels the destruction of the trypsin inhibitors in soybeans. However, the soy lectin appears to be more resistant to inactivation by dry heat treatment (91).

Lectins have for a long time attracted the attention of food scientists and nutritionists because some of these proteins, such as ricin from the castor bean, are toxic to animals. The ability of soybean lectins to inhibit the growth of rats was first demonstrated by Liener (92) who showed that lectin accounted for about 25% of the growth inhibition produced by raw soybeans. Liener (22) reported that animal studies showed that soybean lectin was linked to many health issues such as enlargement of the pancreas, lowering of blood insulin levels, inhibition of the disaccharidase and proteases in the intestines, degenerative changes in the liver and kidneys, and interference with absorption of nonheme iron and lipid from the diet.

A new interest regarding the antitumor effect of lectin arose after first discovery by Aub and others (93) that plant lectin could distinguish between malignant and normal cells and that the difference was on the surface of the cells. Evidence is now emerging that plant lectins possess antitumor activity (i.e., an inhibitory effect on tumor growth) and anticarcinogenic activity (i.e., an inhibitory effect on the induction of cancer by carcinogens). This is supported by both *in vitro* and *in vivo* studies. Evidence also shows that plant lectins may be dynamic contributors to tumor cell recognition, cell adhesion and localization, signal transduction across membranes, mitogenic cytotoxicity, and apoptosis. A review paper is available on the subject (94). Due to their specific properties, lectins are used as a tool for both analytical and preparative purposes in biochemistry, cellular biology, and immunology, as well as for diagnostic and therapeutic purposes in cancer research (95).

Bioactive Peptides

Bioactive peptides occur naturally and are produced during processing (such as fermentation or hydrolysis). Some of these peptides are resistant to digestion and can act as physiological modulators of body functions, and have been found to exert many therapeutic effects, including antiaging, anticancer, and antihypertensive.

The researchers at the University of California, Berkeley, reported the presence of a naturally occurring peptide, lunasin, in soybeans. Lunasin is a unique 43-amino-acid soybean peptide that contains a number of unique characteristics at the carboxyl end: (a) nine Asp (D) residues, (b) an Arg-Gly-Asp (RGD) cell adhesion motif, and (c) a predicted helix with structural homology to a conserved region of chromatin-binding proteins. Lunasin was first isolated from midmaturation soybean seed.

Basically, lunasin is a 2S albumin, also known as Gm2S-1. The small subunit peptide of Gm2S-1 (lunasin) arrests mitosis, leading to cell death when the *lunasin* gene is transected and expressed inside mammalian cells. The antimitotic effect of lunasin is attributed to the binding of a polyaspartyl carboxyl end to regions of hypoacetylated chromatin, similar to that found in centromeres. As a result, the kinetochore complex does not form properly, and the microtubules fail to attach to the centromeres, leading to mitotic arrest and eventually to cell death (96,97). Further studies show that lunasin has a strong anticancer effect (98). A U.S. patent for compositions and methods for delivering effective amounts of lunasin as nutraceuticals was issued in 2002 (99).

Based on a recent study (25) using a Tris-HCl buffer as an extractant and ELISA test, lunasin concentration in commercial soybean cultivars ranged from 0.33–0.95 g/100 g defatted flour, although a wider range of lunasin concentration exists within the exotic germplasm (0.1–1.33 g/100 g defatted soy flour).

References

1. Wang, X.L., *et al.*, *Zhong Guo Da Dou Zhi Ping* [Chinese Soybean Products], Zhong Guo Qing Gong Ye Chubanshe [China Light Industry Publisher], Beijing, China, 1997.
2. Soyatech, Inc., *Soya & Oil Bluebook*, Bar Harbor, Maine, 2004.
3. Kauffman, H.E. (Ed.), *Proceedings of World Soybean Research Conference VI, Global Soy Forum*, Chicago, August 4–7, 1999.
4. ISPUC-III, *Proceedings of the Third International Soybean Processing and Utilization Conference*, Tsukuba, Japan, October 15–20, 2000.
5. Liu, K.S., H. Kauffman, J.Y. Gai, R. Tschang, N. Zhou, and Y. Yu (Eds.), *Proceedings of China & International Soy Conference and Exhibition*, Chinese Cereals and Oils Society, Beijing, China, November 6–9, 2002.
6. Mascardi, F., L.B. Hoffman-Campo, O.F. Saraiva, P.R. Galerani, F.C. Krzyzanowski, and M.C. Carrao-Panizzi, *Proceedings of the VII World Soybean Research Conference, IV International Soybean Processing and Utilization Conference, and III Brazilian Soybean Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004.
7. Orf, J.H., Modifying Soybean Composition by Plant Breeding, in *Proceedings: Soybean Utilization Alternatives*, edited by L. McCann, University of Minnesota, St. Paul, February 16–18, 1988, p. 131.
8. Liu, K.S., F.T. Orthoefer, and E.A. Brown, Association of Seed Size with Genotypic Variation in the Chemical Constituents of Soybeans, *J. Am. Oil Chem. Soc.* 72:189–192 (1995).
9. Han, Y., C.M. Parsons, and T. Hymowitz, Nutritional Evaluation of Soybeans Varying in Trypsin Inhibitor Content, *Poultry Sci.* 70:896–906 (1991).
10. Hammond, E.G., and B.A. Glatz, Biotechnology Applied to Fats and Oils, *Food Biotechnology* 2:173–217 (1988).
11. Liu, K.S., *Soybeans: Chemistry, Technology, and Utilization*, Kluwer Academic Publishers, New York, 1999.
12. Fehr, W.R., and C.F. Curtiss, Breeding for Fatty Acid Composition of Soybean Oil, in *Proceedings of the VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 815–821.

13. Hymowitz, T., F.I. Collins, J. Panczner, and W.M. Walker, Relationship between the Content of Oil, Protein, and Sugar in Soybean Seed, *Agron. J.* 64:613–616 (1972).
14. Taylor, N.B., R.L. Fuchs, J. MacDonald, A.R. Shariff, and S.R. Padgett, Compositional Analysis of Glyphosate-Tolerant Soybeans Treated with Glyphosate, *J. Agric. Food Chem.* 47:4469–4473 (1999).
15. Fernando, S.M., and P.A. Murphy, HPLC Determination of Thiamine and Riboflavin in Soybeans and Tofu, *J. Agric. Food Chem.* 38:163–167 (1990).
16. Guzman, G.J., and P.A. Murphy, Tocopherols of Soybean Seeds and Soybean Curd (Tofu), *J. Agric. Food Chem.* 34:791–795 (1986).
17. Coward, L., N.C. Barnes, K.D.R. Setchell, and S. Barnes, Genistein, Daidzein, and Their Beta-Glycoside Conjugates: Antitumor Isoflavones in Soybean Foods from American and Asian Diets, *J. Agric. Food Chem.* 41:1961–1967, 1993.
18. Wang, H.-J., and P.A. Murphy, Isoflavone Composition of American and Japanese Soybeans in Iowa: Effects of Variety, Crop Year and Location, *J. Agric. Food Chem.* 42:1674–1677 (1994).
19. Ardit, T., T. Meredith, and P. Flowerman, Renewed Interest in Soy Isoflavones and Saponins, *Cereal Food World* 45:414–417 (2000).
20. Lolos, G.M., N. Palamidis, and P. Markakis, The Phytic Acid-Total Phosphorus Relationship in Barley, Oats, Soybeans, and Wheat, *Cereal Chem.* 53:876 (1976).
21. Rao, A.V., and S.A. Janezic, The Role of Dietary Phytosterols in Colon Carcinogenesis, *Nutr. Cancer* 18:43–52, 1992.
22. Liener, I.E., Implications of Antinutritional Components in Soybean Foods, *Crit. Rev. Food Sci. Nutr.* 34:31–67 (1994).
23. Anderson, R.L., and W.J. Wolf, Compositional Changes in Trypsin Inhibitors, Phytic Acid, Saponins, and Isoflavones Related to Soybean Processing, *J. Nutr.* 125:581S–588S (1995).
24. Padgett, S.R., N.B. Taylor, D.L. Nida, M.R. Bailey, J. MacDonald, L.R. Holden, and R.L. Fuchs, The Composition of Glyphosate-Tolerant Soybean Seeds is Equivalent to That of Conventional Soybeans, *J. Nutr.* 126:702–716 (1996).
25. de Mejia, E.G., W. Wang, M. Vasconez-Costa, R. Nelson, and B.O. de Lumen, Physiologically Active Peptides in Soybean and Soy Products, in *Proceedings of the VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 775–779.
26. U.S. Department of Agriculture Nutrient Data Laboratory Website, Nutrient Database for Standard Reference, Release 13, available at www.nal.usda.gov/fnic/foodcomp/ (accessed June 16, 2004).
27. Messina, M., V. Messina, and K.D.R. Setchell, *The Simple Soybean and Your Health*, Avery Publishing Group, Garden City Park, New York, 1994.
28. Carroll, K.K., and E.M. Kurowska, Soy Consumption and Cholesterol Reduction: Review of Animal and Human Studies, *J. Nutr.* 125:594S–597S (1995).
29. Wang, C.Y., and R. Wixon, Phytochemicals in Soybeans and Their Potential Health Benefits, *INFORM* 10(4):315–321 (1999).
30. Anthony, M.S., Soy and Cardiovascular Disease: Cholesterol Lowering and Beyond, *J. Nutr.* 130:662S–663S (2000).
31. Friedman, M., and D.L. Brandon, Nutritional and Health Benefits of Soy Proteins, *J. Agric. Food Chem.* 49:1069–1086 (2001).

32. Messina M., Potential Public Health Implications of the Hypcholesterolemic Effects of Soy Protein, *Nutr.* 19:280–281 (2003).
33. Nielsen, N.C., Structure of Soy Proteins, in *New Protein Foods, Vol. 5. Seed Storage Proteins*, edited by A.M. Altschul and H.L. Wilcke, Academic Press, Orlando, Florida, pp. 27–64.
34. Nishizawa, N.K., S. Mori, Y. Watanabe, and H. Hirano, Ultrastructural Location of the Basic 7S Globulin in Soybean (*Glycine max*) cotyledons. *Plant Cell Physiol.* 35:1079–1085 (1994).
35. Zarkadas, C.G., Z. Yu, H.D. Voldeng, and A. Minero-Amador, Assessment of the Protein Quality of a New High-Protein Soybean Cultivar by Amino Acid Analysis, *J. Agric. Food Chem.* 41:616–623 (1993).
36. Food and Agriculture Organization/World Health Organization, *Protein Quality Evaluation. FAO/WHO Nutrition Meetings, Report Series 51*, Author, Rome, 1990.
37. Sarwar, G., The Protein Digestibility-Corrected Amino Acid Score Method Overestimates Quality of Proteins Containing Antinutritional Factors and of Poorly Digestible Proteins Supplemented with Limiting Amino Acids in Rats, *J. Nutr.* 127:758–764 (1997).
38. Schaafsma, G., The Protein Digestibility-Corrected Amino Acid Score, *J. Nutr.* 130:1865S–1867S (2000).
39. Anderson, J.W., B.M. Johnstone, and M.L. Cook-Newell, Meta-analysis of the Effects of Soy Protein Intake on Serum Lipids, *N. Engl. J. Med.* 333:276 (1995).
40. Food and Drug Administration, Food Labeling, Health Claims, Soy Protein, and Coronary Heart Disease, *Fed. Reg.* 57:699–733 (1999).
41. Jenkins, D.J., C.W. Kendall, D. Faulkner, *et al.*, A Dietary Portfolio Approach to Cholesterol Reduction: Combined Effects of Plant Sterols, Vegetable Proteins, and Viscous Fibers in Hypercholesterolemia, *Metabolism* 51:1596–604 (2002).
42. Erdman, J.W., Soy Protein and Cardiovascular Disease: A Statement for Healthcare Professionals from the Nutrition Committee of AHA, *Circulation* 102:2555–2559 (2000).
43. Moriyama, T., K. Kishimoto, K. Nagai, R. Urade, T. Ogawa, S. Utsumi, N. Maruyama, and M. Maebuchi, Soybean Beta-Conglycinin Diet Suppresses Serum Triglyceride Levels in Normal and Genetically Obese Mice by Induction of Beta-Oxidation, Down Regulation of Fatty Acid Synthase, and Inhibition of Triglyceride Absorption, *Biosci. Biotechnol. Biochem.* 68:352–359 (2004).
44. Stephenson, T.J., J.W. Anderson, D.J. Jenkins, C. Kendall, and P. Fanti, Beneficial Effects of Soy Protein Use on Renal Function in Young Type I Diabetic Subjects with Early Diabetic Nephropathy, *J. Nutr.* 132:585S (2002).
45. Pedrini, M.T., A.S. Levey, J. Lau, T.C. Chalmers, and P.H. Wang, The Effect of Dietary Protein Restriction on the Progression of Diabetic and Nondiabetic Renal Diseases: A Meta-analysis, *Ann. Intern. Med.* 124:627–632 (1996).
46. Watkins, T.R., K. Pandya, and O. Mickelsen, Urinary Acid and Calcium Excretion. Effect of Soy versus Meat in Human Diets, in *Nutritional Bioavailability of Calcium*, edited by C. Kies, American Chemical Society, Washington, D.C., 1985.
47. Spence, L.A., E.R. Lipscomb, J. Cadogan, B.R. Martin, M. Peacock, and C.M. Weaver, Effects of Soy Isoflavones on Calcium Metabolism in Postmenopausal Women, *J. Nutr.* 132:581S (2002).
48. Liu, K.S., Modifying Soybean Oil through Plant Breeding and Genetic Engineering, in *World Oilseed Conference Proceedings*, edited by R.L. Wilson, AOCS Press, 2001, pp. 84–89.

49. Chapkin, R.S., Reappraisal of the Essential Fatty Acids, in *Fatty Acids and Their Health Implications*, edited by C.K. Chow, Marcel Dekker, New York, 1992, Chapter 18, pp. 429–435.
50. Martin, M.J., S.B. Hulley, W.S. Browner, L.H. Kuller, and D. Wentworth, Serum Cholesterol, Blood Pressure, and Mortality: Implications from a Cohort of 361,662 Men, *Lancet* 2:933–936 (1986).
51. Chow, C.K. (Ed.), *Fatty Acids and Their Health Implications*, Marcel Dekker, New York, 1992.
52. Cristofaro, E., F. Mottu, and J.J. Wuhrmann, Involvement of the Raffinose Family of Oligosaccharides in Flatulence, in *Sugar in Nutrition*, edited by H.L. Sipple and K.W. McNutt, Academic Press, New York, 1974, Chapter 20.
53. Burkitt, D.P., and H.C. Trowell (Eds.), *Refined Carbohydrate Foods and Disease, Some Implications of Dietary Fiber* [Monograph], Academic Press, London, UK, 1975.
54. Vahouny, G., and D. Kritchevsky (Eds.), *Dietary Fibers Basic and Clinical Aspects*, Plenum Press, New York, 1986.
55. Olson, A., G.M. Gray, and M.-C. Chiu, Chemistry and Analysis of Soluble Dietary Fiber, *Food Technol.* Feb. 41:71–80 (1987).
56. Masai, T., K. Wada, K. Hayakawa, I. Yoshihara, and T. Mitsuoka, Effects of Soybean Oligosaccharides on Human Intestinal Flora and Metabolic Activities, *Japan J. Bacteriol.* 42:313 (1987).
57. Tomomatsu, H., Health Effects of Oligosaccharides, *Food Technol.* Oct. 48:61–65 (1994).
58. Bates, R.P., and R.F. Matthews, Ascorbic Acid and beta-Carotene in Soybeans as Influenced by Maturity, Sprouting, Processing and Storage, *Proc. Fla. State Hort. Soc.* 88:266–271 (1975).
59. Pryde, E.H., Composition of Soybean Oil, in *Handbook of Soy Oil Processing and Utilization*, edited by S.R. Erickson, E.H. Pryde, O.L. Brekke, T.L. Mounts, and R.A. Falb, American Oil Chemists' Society, Champaign, Illinois, 1980, p. 13.
60. O'Dell, B.L., Effect of Soy Protein on Trace Mineral Availability, in *Soy Protein and Human Nutrition*, edited by H.L. Wilcke, D.R. Hopkins, and D.H. Waggle, Academic Press, New York, 1979.
61. Zeisel, S., and J. Blusztain, Choline and Human Nutrition, *Annu. Rev. Nutr.* 14:269–296 (1994).
62. Wald, N.J., C. Hilary, M.R.L. Watt, G.W. Donald, M. Joseph, and M.S. John, Homocysteine and Schemic Heart Disease. Results of a Prospective Study with Implications Regarding Prevention, *Arch. Intern. Med.* 158:862–867 (1998).
63. Ghoshal, A., and E. Farber, Choline Deficiency, Lipotrope Deficiency, and the Development of Liver Disease including Liver Cancer: A New Perspective, *Lab Invest.* 68:255–258 (1993).
64. Kneuchel, F., Lecithin Increases Plasma Free Choline and Decreases Hepatic Steatosis in Long-Term Parenteral Nutrition Patients, *Gastroenterology* 102: 1363–1370 (1979).
65. Ladd, S.L., S.A. Sommer, S. LaBerge, and W. Toscano, Effect of Phosphalidylcholine on Explicit Memory, *Clin. Neuropharmacol.* 16:540–549 (1993).
66. Eldridge, A., and W. Kwolek, Soybean Isoflavones: Effect of Environment and Variety on Composition, *J. Agric. Food Chem.* 31:394–396 (1983).

67. Kudou, S., Y. Fleury, D. Welti, D. Magnolato, T. Uchida, K. Kitamura, and K. Okubo, Malonyl Isoflavone Glycosides in Soybean Seeds (*Glycine max* Merrill), *Agric. Biol. Chem.* 55:2227–2233 (1991).
68. Coward, L., M. Smith, M. Kirk, and S. Barnes, Chemical Modification of Isoflavones in Soyfoods during Cooking and Processing, *Am. J. Clin. Nutr.* 68:1496S–1491S, 1998.
69. Zubik, L., and M. Meydani, Bioavailability of Soybean Isoflavones from Aglycone and Glucoside Form in American Women, *Am. J. Clin. Nutr.* 77:1459–1465 (2003).
70. Lipkin, R., The Health Benefits of Saponins, *Sci. News* Dec. 9, 1995.
71. Law, M., Plant Sterol and Stanol Margarines and Health, *Brit. Med. J.* 320:861–864 (2000).
72. Piironen, V., D.G. Lindsay, T.A. Miettinen, J. Toivo, and A.M. Lampi, Review: Plant Sterols: Biosynthesis, Biological Function and Their Importance to Human Nutrition, *J. Sci. Food Agri.* 80:939–966 (2000).
73. Zawistowski, J., and D.D. Kitts, Sterols from Soybeans and Other Sources in Cholesterol Reduction, in *Proceedings of the VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 906–912.
74. Pernollet, J.-C., Protein Bodies of Seeds: Ultrastructure, Biochemistry, Biosynthesis and Degradation, *Phytochemistry* 17:1473–1480 (1978).
75. Maga, J.A., Phytate: Its Chemistry, Occurrence, Food Interactions, Nutritional Significance, and Methods of Analysis, *J. Agric. Food Chem.* 30:1–9 (1982).
76. Weaver, C.M., N. Nelson, and J.G. Elliott, Bioavailability of Iron to Rats from Processed Soybean Fractions Determined by Intrinsic and Extrinsic Labeling Techniques, *J. Nutr.* 114:1042–1048 (1984).
77. Young, V.R., and M. Janghorbani, Soy Proteins in Human Diets in Relation to Bioavailability of Iron and Zinc: A Brief Review, *Cereal Chem.* 58:12 (1981).
78. Sandberg, A.S., N.G. Carlsson, and U. Svanberg, Effect of Inositol, Tri-, Tetra-, Penta-, and Hexaphosphates on in Vitro Estimation of Iron Availability, *J. Food Sci.* 54:159–161 (1989).
79. Vaintraub, I.A., and V.P. Bulmaga, Effect of Phytate on the in Vitro Activity of Digestive Enzymes, *J. Agric. Food Chem.* 39:859 (1991).
80. Chen, B.H.-Y., and C.V. Morr, Solubility and Forming Properties of Phytate-Reduced Soy Protein Isolate, *J. Food Sci.* 50:1139–1142 (1985).
81. Shamsuddin, A.M., Anti-cancer Function of Phytic Acid, *Int. J. Food Sci. Technol.* 37:769–782 (2002).
82. Liu, K.S., and P. Markakis, An Improved Colorimetric Method for Determining Antitryptic Activity in Soybean Products, *Cereal Chem.* 66:415–422 (1989).
83. Greene, G.M., and R.L. Lyman, Feedback Regulation of Pancreatic Enzyme Secretion in Rats, *Proc. Sci. Exp. Biol. Med.* 140:6–12 (1972).
84. Kennedy, A.R., Overview: Anticarcinogenic Activity of Protease Inhibitors, in *Protease Inhibitors as Cancer Chemopreventive Agents*, edited by W. Troll and A.R. Kennedy, Plenum Publishing, New York, 1993, pp. 9–64.
85. Kennedy, A.R., The Bowman-Birk Inhibitor from Soybeans as an Anticarcinogenic Agent, *Am. J. Clin. Nutr.* 68:1406S–1412S (1998).
86. Meyskens, F.L., Jr., Development of Difluoromethyl Ornithine and Bowman-Birk Inhibitor as Chemopreventive Agents by Assessment of Relevant Biomarker Modulation: Some lessons Learned, *IARC Sci. Pub.* 154:49–55 (2001).

87. Kennedy, A.R., and B.F. Szuhaj, Bowman-Birk Inhibitor Concentrate Compositions and Methods for the Treatment of Pre-malignant Tissue, U.S. Patent 5,505,946, April 9, 1996.
88. Pulsztai, A., *Plant Lectins*, Cambridge University Press, Cambridge, UK, 1991.
89. Lotan, R.H., W. Sieggelman, H. Lit, and N. Sharon, Subunit Structure of Soybean Agglutinin, *J. Biol. Chem.* 249:1219 (1974).
90. de Mejia, E.G., M. Vasconez, and R. Nelson, Concentration of Lectins in Soybean Seeds, in *Abstracts and Contributed Papers and Posters for VII World Soybean Research Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, p. 140.
91. Calderon de la Barca, A.M., L. Vazquez-Moreno, and M.R. Robles-Burgueno, Active Soybean Lectin in Foods: Isolation and Quantitation, *Food Chem.* 39:321 (1991).
92. Liener, I.E., Soyin, a Toxic Protein from the Soybean. I. Inhibition of Rat Growth, *J. Nutr.* 49:527 (1953).
93. Aub, J.C., C. Tieslau, and A. Lankester, Reaction of Normal and Tumor Cell Surfaces to Enzymes. I. Wheat Germ Lipase and Associated Mucopolysaccharides, *Proc. Natl. Acad. Sci. USA* 50:613–619 (1963).
94. Abdullaev, F.I., and E.G. de Mejia, Antitumor Effect of Plant Lectins, *Nat. Toxins* 5:157–163 (1997).
95. Mody, R., S. Joshi, and W. Chaney, Use of Lectins as Diagnostic and Therapeutic Tools for Cancer, *J. Pharmacol. Toxicol. Methods* 33:1–10 (1995).
96. Galvez, A.F., M.J.R. Revilla, and B.O. de Lumen, Novel Methionine-Rich Protein from Soybean Cotyledon: Cloning and Characterization of cDNA (Accession No. AF005030, Plant Gene Register #PGR97-103), *Plant Physiol.* 114:1567–1569 (1997).
97. Galvez, A.F., and B.O. de Lumen, A Soybean cDNA Encoding a Chromatin-Binding Peptide Inhibits Mitosis of Mammalian Cells, *Nat. Biotech.* 17:495–500 (1999).
98. Galvez, A.F., N. Chen, J. Macasieb, and B.O. de Lumen, Chemopreventive Property of a Soybean Peptide (Lunasin) that Binds to Deacetylated Histones and Inhibits Acetylation, *Cancer Res.* 61:7473–7478 (2001).
99. de Lumen, B.O., and A.F. Galvez, Soybean Protein Nutraceuticals, U.S. Patent 6,391,848, May 21, 2002.

Chapter 2

Edible Soybean Products in the Current Market

KeShun Liu

University of Missouri, Columbia, MO 65211

For thousands of years, the Chinese and people in neighboring countries have consumed soybeans in various forms of traditional soyfoods, such as tofu, soy sauce, miso (*jiang* in China), soy sprouts, and vegetable soybeans (Fig. 2.1). Soyfoods are among the most popular foods in the Far East. Yet, until recently, soyfoods had never been common in Western diets. Despite its rich history as a food, its unique features as a crop, and increasing annual production, the soybean had suffered a severe image problem in the West because of its unfamiliar flavor (commonly described as beany). One approach that was taken to overcome the poor image of soy was to market soy products without using the word “soy.” Thus, soy oil became “vegetable oil,” and soy burgers became “veggie burgers” or “harvest burgers.” Consequently, most of the soybean production in the United States is crushed into oil and defatted meal (Fig. 2.2). Although soybean oil is produced almost entirely for human consumption, soy meal is mainly used as animal feed. Only a small portion of defatted meal is processed into soy protein products for human consumption by modern processing technology. These processed soy products are not consumed directly but are incorporated as ingredients in various types of Western food.

The past one and a half decades have been a turning point for the soyfoods industry in the United States. According to Golbitz (1), the U.S. soyfoods market is



Figure 2.1. Traditional soyfoods. Courtesy of United Soybean Board.



Figure 2.2. Soy flour and defatted meal after crushing.

one of the fastest-growing categories in the food industry. Retail sales increased from \$852 million in 1992 to \$3.65 billion in 2002 and are projected to \$4.0 billion in year 2004 (Fig. 2.3). The annual growth rate averaged 14% for the years 1992–2002, with some categories, such as soymilk, meat alternatives, and energy bars, growing at an even faster rate.

One of the major forces that drive soyfood market growth and consumer interest in using soy as food has been the medical discovery about the health benefits of soy. For many years, soybeans had been primarily identified with their high protein and oil content. Yet, for the past one and half decades there has been much interest among medical researchers in studying the health benefits of direct human consumption of soybeans as food. Thousands of studies have been conducted, and many are ongoing, to discover the role of soyfoods in preventing and treating chronic diseases. Epidemiological human as well as animal studies have shown that soyfoods can reduce the incidence of breast, colon, and prostate cancers; heart disease; osteoporosis; and menopausal symptoms (2–9). Among the many soy components examined, soy protein and isoflavones exhibit

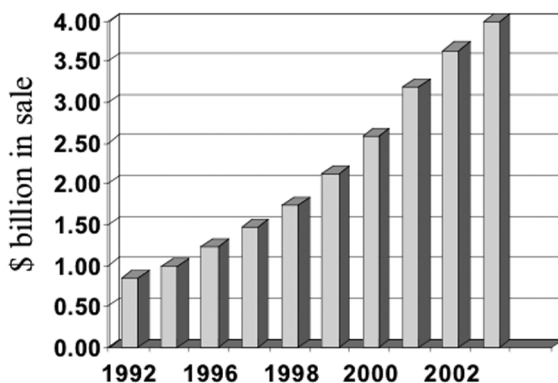


Figure 2.3. U.S. soyfood sales since 1992. Data adapted from Golbitz (1).

the most promise as the source of the health benefits of soy (4–6). These findings about the health benefits of soy have become a powerful message for improving the image of soy as food, increasing consumer interest in soyfoods and soy-enriched foods, and spurring production and sales of these food products.

The previous chapter explained why soybeans are a powerhouse of nutrients and phytochemicals by describing each chemical constituent in soybeans with respect to chemistry, occurrence, and current medical findings. Yet, unlike rice, soybeans are not made palatable by a simple cooking procedure. Thus, in order for the general public to reap the health benefits, the important task facing the food industry as well as the scientific community is to produce soy food products that are tasty, available, and acceptable to consumers so that soyfoods can become a major component of Western diets. Although some health-promoting components, such as isoflavones, have been made into pills, the ultimate and efficient approach for delivering healthy soy into the human body is apparently through regular consumption as food.

Fortunately, the soybean is so versatile that it can be processed into a wide variety of food products. Advancements in processing (10–12) and breeding technology (Chapter 14) plus human creativity have further increased the versatility of soy food products. Generally speaking, soyfoods in the current U.S. and global markets can be classified into six major groups: soy oil, traditional soyfoods, soy protein products, modern soyfoods, soy-enriched foods, and functional soy ingredients/dietary supplements. Table 2.1 lists various soyfoods within the six categories, and [Figure 2.4](#) gives a general outline of the processing of soybeans into various soy food products.

TABLE 2.1

Classification of Various Edible Soy Products in the Current Market

Category	Product Examples
Traditional soyfoods	Soymilk, tofu, soy sprouts, yuba, green vegetable soybeans
Soy oil products	Salad and cooking oil, shortening, margarine
Soy protein products	Soy flour, concentrate, isolate, textured soy proteins
Modern soyfoods	Soy burgers, tofu burgers, soy sausages, soy chicken nuggets Soymilk, soy ice cream, soy yogurt
Soy-enriched foods	Bakery products: soy bread, soy pasta Meat products: sausages, hamburgers Dairy products: ice cream, yogurt, juice-soymilk or milk-soymilk blends
Soy dietary supplements and nutraceuticals	Soy isoflavones, lecithin, vitamin E, sterols, oligosaccharides, soy peptides

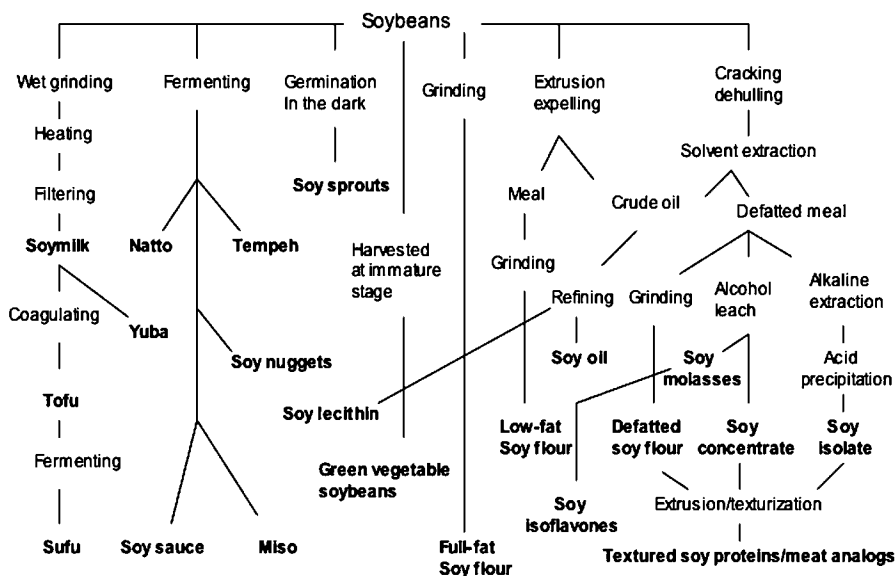


Figure 2.4. General flow chart of processing soybeans into various edible products.

This chapter provides a brief overview of various types of soyfoods and ingredients found in the current food market. It deals with the important issue of how we can reap the health benefits of soy through making and consuming various soy products. Detailed information on soy food products and their processing can be found in Shurtleff and Aoyagi (13), Wang *et al.* (14), Liu (15), and Hui *et al.* (16).

Soybean Oil

As a commodity, soybeans are regarded as an oilseed crop. A major portion of annual soybean production is crushed for oil and meal. In the United States, soybean oil is a leading edible oil, constituting about 80% of the total annual consumption of edible fats and oil. The large-volume usage of soybean oil within the United States and the widening acceptance of the oil in other parts of the world have been attributed to at least three factors: (a) a plentiful and dependable supply, (b) a competitive price, and (c) the improvements made in the flavor and oxidative stability of the oil through advanced processing and breeding technology. In addition, the large-volume usage of soy meal as animal feed serves as another driving force for increased production of soybeans and subsequently of soy oil.

When compared with the majority of other vegetable oils, crude soybean oil has the following unique physicochemical features: (a) It has a relatively high content of phospholipids that must be removed by a process known as degumming; the recovered gums are the source of commercial lecithin. (b) It has a high level of unsaturation and therefore remains liquid over a relatively wide temperature range. (c) It has

a relatively high content of linolenic acid (7–10%), which makes it susceptible to oxidation and flavor reversion. (*d*) It has a tendency to form β crystals during crystallization. (*e*) Crude soy oil contains naturally occurring antioxidants such as tocopherols, which are not completely removed during processing.

Because of these unique features, for soybean oil to have improved flavor stability as well as different consistency for a wide variety of edible applications, the oil is normally subjected to several steps of processing prior to its end application, including degumming, alkaline refining, bleaching, and deodorizing to remove impurities (phospholipids, trace metals, soaps, etc.). For many applications, one or more additional processing steps, such as hydrogenation, winterization, or transesterification, are also needed to improve the soy oil's physical characteristics as well as its oxidative stability.

A wide variety of products based on edible fats and oils are available in the consumer market. Salad and cooking oils, shortening, margarine, mayonnaise, salad dressings, and confectionery coatings are some of the widely available products. These products are either based entirely on fats and oils or contain fat or oil as a principal ingredient. Many of these products are also sold in commercial quantities to food processors, snack food manufacturers, bakeries, restaurants, and institutions. Advancements in refining and post-refining processes have made soybean oil a versatile high-quality oil for making almost every commercial oil product just mentioned. The subject of soybean oil processing and application is covered more thoroughly in the literature (15,17,18).

Furthermore, for the past three decades, plant breeding and biotechnology have been used to change the fatty acid composition of soybean oil, resulting in several types of soybeans oils with improved functionality, stability, and/or nutritional quality for specific end uses. Examples include low-linolenic, high-linoleic, and low-saturate soy oils. The result has been further expansion of soy oil uses as food along with an improvement in oil quality with minimal environmental impact (19,20).

Traditional Soyfoods

Traditional soyfoods, also known as Oriental soyfoods, originated in China and other Far East countries hundreds or even thousands of years ago (Fig. 2.1). They remain popular today. Almost all traditional soyfoods are made from whole soybeans. They can be classified into two categories: nonfermented and fermented. Nonfermented soyfoods include soymilk, tofu, soy sprouts, soymilk film (yuba), soynuts, green vegetable soybeans, and others. Fermented soyfoods include soy sauce, miso, tempeh, natto, and others. Traditional soyfoods that are commonly seen in the U.S. market include soy sauce, tofu, soymilk, tempeh, green vegetable soybeans, soynuts, and soy sprouts.

Nonfermented Soyfoods

Nonfermented soyfoods are by far the largest volume of traditional soyfood production. Unlike some fermented soyfoods that serve as seasoning, nonfermented soyfoods are almost all for nourishment.

Soymilk. Soymilk is a water extract of soybeans, resembling dairy milk in appearance and composition. It is widely believed that soymilk, along with tofu, was first made in China during the Han Dynasty in the second century BC.

Based on the method of preparation, soymilk is generally divided into traditional soymilk and modern soymilk. Traditional soymilk, known as *dou jiang* in Chinese, is made by a thousand-year-old method in the home or on the village level. The procedure includes soaking, grinding, filtering, and heating (Fig. 2.5). Considered an intermediate product during tofu production, *dou jiang* is generally served fresh and hot during breakfast. The product not only has a limited shelf life, but also possesses a characteristic beany flavor and bitter or astringent taste, with all nutrients coming solely from original soybeans.

In contrast, modern soymilk, sometimes referred to as soy beverage or soy drink, is produced by the use of modern technology and equipment to maximize taste, flavor, nutritional value, and convenience. The techniques used by modern manufacturers may include but are not limited to beany flavor reduction, decantation, formulation, fortification, homogenization, ultra-high-temperature processing (21), aseptic packaging, and automation. Known as *dou ru* or *dou nai* in Chinese, modern soymilk has a relatively bland taste with its own commercial identity and standards. In most cases it is flavored, sweetened, and/or fortified for better taste and better nutrition, and packed for longer shelf life, as compared with traditional soymilk. It may also be in a powdered or condensed form. Consequently, a wide array of soymilk products is seen in the market, with different terms describing the products, ranging from soymilk to soy beverage, and from soy drink to dairy alternative. Based on solids concentration, we have light, dairylike, and rich soymilk. With respect to formulation, we have plain and sweetened, and original and flavored soymilk. With respect to fortification, we have regular, enriched, and blended soymilk. We also have refrigerated and

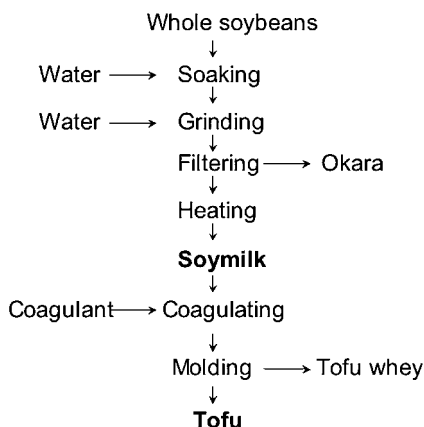


Figure 2.5. A traditional Chinese method for making soymilk and tofu.

nonrefrigerated products. In the U.S. market, aseptically packaged soymilk has been popular, as it requires no refrigeration. Yet, in recent years, refrigerated types are gaining popularity, as the products are sold alongside dairy milk (1,22).

In general, soymilk has total solids of 8–10%, depending on the water:bean ratio in its processing. Among the solids, protein constitutes about 3.6%; fat, 2.0%; carbohydrates, 2.9%; and ash, 0.5%. Thus, the soymilk composition compares favorably with those of cow's milk and human milk. The noticeable differences are that (a) soymilk is cholesterol-free and lactose-free and (b) soymilk contains about 0.25 mg/g of total isoflavones on a wet basis or 3.26 mg/g on a dry matter basis, a dry weight value similar to that of raw soybeans (23–25).

As an alternative to dairy milk, soymilk provides nutrients to people in regions where animal milk supply is inadequate. It is especially important for infants and children who exhibit allergic reactions to dairy or human milk. As a beverage, soymilk offers consumers both refreshment and nutrition. Furthermore, in Western society, soymilk offers a healthy choice for people who want to avoid animal proteins and reap the benefits of soy. The problem with soymilk is that most products in the market are heavily formulated with sugar, gums, and flavorings to improve stability and mask beany flavor or impart a new flavor.

In North America, there are about 50 companies commercially producing soymilk. Although most of these products are limited to local distribution, there are a few that have enjoyed considerable expansion in recent years with respect to both production volume and distribution systems. The market for soymilk has grown the fastest among the types of soyfoods, with an annual growth rate anywhere between 20 and 30%. Current soymilk sales in the United States were estimated at \$650 million at the retail level in 2003 (1). For details on soymilk production, refer to Shurtleff and Aoyagi (13), Chen (26), Liu (15), and Imram (27).

Tofu. Tofu is prepared by coagulating traditional soymilk with a coagulant. It can be defined as water-extracted and salt- or acid-precipitated soybase in the form of a curd, resembling a soft white cheese or a very firm yogurt.

Variety and Current Market. For thousands of years, tofu has been the most popular way of consuming soybean as food in China and other Far East countries or regions. It is inexpensive, nutritious, and versatile. It can be served as a meat or cheese substitute, fresh or prepared with virtually any other foods. Most popularly, it is served in soups or separate dishes stir-fried with meat and/or vegetables. It can also be further processed into various secondary tofu products, including deep-fried tofu, grilled tofu, frozen tofu, dried-frozen tofu, and fermented tofu (sufu). In most cases, these processed tofu products have different characteristics, end uses, and commercial identities than the original plain tofu.

In recent years, tofu has become increasingly popular throughout the world, as increased numbers of consumers are looking for healthy foods of plant origin. This has led to increasing development of an infrastructure for large-scale commercial tofu production and distribution. In the United States, sales of tofu have increased

from \$38 million in 1980 to about \$260 million in 2003 (Fig. 2.6). Tofu is sold mainly refrigerated, in different types of packaging, including water-filled tubs, vacuum packs, and aseptic packaging. In the past, tofu and many other soyfoods were available only in natural or Oriental food stores; nowadays, they are sold in most supermarkets.

The new wave of tofus on the Western market includes baked, flavored, and smoked varieties. Basically, tofu is first seasoned and marinated with desired spices, herbs, flavorings, and sauces, and then baked or smoked. Baked tofu, cut into slices or pieces, comes in plastic-wrapped packages ranging from 4 to 8 ounces. These already-seasoned and ready-to-eat tofu products are one of the most convenient soyfoods. Their preparation also effectively masks beany taste and imparts different types of flavoring to suit different tastes, including Italian style, Thai style, Mexican style, Oriental style, Hawaiian, savory, teriyaki, garlic, Szechwan style, and a virtually unlimited variety of others (28).

Nutritional Value and Health Benefits. Tofu is one of the best soyfoods that can deliver health benefits. First, tofu is a nutritious and natural food. It is made of whole soybeans. Nothing is added during processing except for a fractional quantity of food-grade coagulant. On a wet basis, a typical pressed tofu with moisture content in the range of 85% contains about 7.8% protein, 4.2% lipid, and 2 mg/g calcium. On a dry basis, it contains about 50% protein and 27% oil. The remaining components are carbohydrates and minerals (29). Second, the fat content in tofu is basically soy oil in its natural state. Therefore, it is low in saturated fat, and contains almost zero trans fatty acids and zero cholesterol. Third, tofu is a rich source of soy protein. Tofu is among the few whole-bean soyfoods that can carry the FDA-approved health claim because it meets the requirements of the FDA ruling (30): (a) It contains a

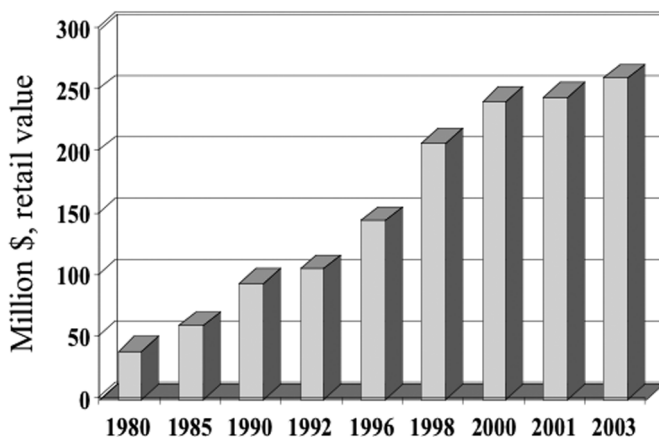


Figure 2.6. U.S. tofu sales since 1980.
Data adapted from Golbitz (22).

minimal of 6.25 g soy protein per serving; (b) it is low in cholesterol and saturated fat; and (c) although tofu is not low in fat content, its fat comes solely from soybeans. Fourth, because tofu is made of whole soybeans, many beneficial phytochemicals are retained after processing, including isoflavones. During tofu processing, there are some losses of isoflavones in whey and okara (31,32), and the chemical form of the isoflavones undergoes some modification as well (24). However, on a dry matter basis, tofu has a total isoflavone content ranging from 2.031 to 3.882 mg/g (23), within the range for raw soybeans. Wakai *et al.* (33) reported that tofu, fried tofu, miso, and natto are the top four foods for 90% of Japanese isoflavone intake.

Finally, tofu is a rich source of calcium. Calcium in tofu comes from two sources: raw soybeans and the use of a common coagulant, calcium sulfate. Of course, some tofu is made using other coagulants such as glucono-delta-lactone and magnesium chloride. In this case, calcium content can be increased through enrichment.

Studies show that increased tofu consumption is linked to reduced risk of several cancers, including breast cancer, colorectal cancer, stomach cancer, and lung cancer (33,34). Tofu consumption also helps alleviate hot flashes in menopausal women (35).

General Processing. At present, throughout many regions, tofu is being made both at home and at commercial plants. Therefore, there are many variations in tofu making to suit making different types of tofu products and using different types of equipment for varying scales of production. Yet, the basic procedure and principle remain similar to the traditional Chinese method developed some 2,000 years ago. Basically, the procedure starts with preparation of soymilk by soaking, rinsing, and grinding whole soybeans into a slurry, followed by filtering the slurry to separate the residue, and cooking the soy extract to make it edible (Fig. 2.5). The details of the seven basic steps of the tofu-making process are the following:

1. *Soaking.* Dry whole soybeans, preferably beans with large seed size and light hilum, are cleaned, measured (or weighed), and soaked in water overnight. The volume of water is normally about 2–3 times the bean volume.
2. *Draining and rinsing.* The soaked beans are drained and rinsed with fresh water 2–3 times.
3. *Grinding.* The wet, clean soybeans are ground in a mill with addition of fresh water. The water:bean ratio is normally in the range of 6:1 to 10:1. The slurry is collected in a big container.
4. *Filtering.* The bean slurry is filtered through a screen, cloth, or pressing sack. The residue, known as soy pulp or okara, is removed. It is normally washed once or twice with water (cold or hot), stirred, and re-pressed to maximize milk yield. The total volume of the combined filtrate (raw soymilk) is about 6–10 times the original bean volume.
5. *Cooking.* The raw milk is now heated until boiling and maintained at this temperature for 5–10 min. To avoid burning the milk at the bottom of the cooking vessel,

slow heating with frequent stirring is necessary. In commercial production, a double boiler or a heat exchanger is commonly used. Alternatively, soy slurry may be heated before filtering into soymilk. This procedure is particularly popular in Japan.

6. *Coagulating.* After the milk is heated, it is transferred to another container. At the same time, a coagulant suspension is prepared by mixing a powdered coagulant with some hot water. The most commonly used coagulant is calcium sulfate; glucono-delta-lactone (GDL) and magnesium chloride are also commonly used. After the coagulant is added, the mixture is allowed to stand for about 20–30 min for coagulation to complete.
7. *Molding.* The soy curd thus formed is now ready for molding. It is first broken by stirring, and then transferred to a shallow forming box lined with cloths at each edge. As whey is pressed out, tofu curd becomes firm. Cooled tofu is finally cut into cakes, which are ready to be served or immersed in cold water for short storage or sale at local markets. Keep in mind that some tofu is made without the pressing stage, such as silken tofu and lactone tofu.

Based on the procedure just described, tofu making is similar to cheese making in some aspects. Both involve protein coagulation and whey removal. The difference is that tofu is made out of soymilk whereas cheese is made out of dairy milk. Another difference is that in cheese making, we often use rennet, but in tofu making, we use a salt to precipitate protein. Detailed coverage of tofu production and quality factors can be found in Shurtleff and Aoyagi (13) and Liu (15).

Soymilk Film (Yuba). Yuba is another soyfood derived from soymilk. It is a creamy yellow, bland-flavored protein-lipid film, varying from fresh to semidried or dried. Named after a Japanese word for soymilk film, yuba is also known as dried bean curd in English, and as *dou fupi* or *fuzhu* in Chinese.

To make yuba, one needs to first make a rich soymilk. The soymilk is then heated in a flat, open pan to near boiling temperature (85–95°C). A film gradually forms on the liquid surface due to surface dehydration. After the film becomes toughened it can be lifted with two sticks or by passing a rod underneath it. The film is hung on a line or spread on a galvanized wire mesh for drying.

Typical dry yuba consists of 55% protein, 28% lipids, 12% carbohydrates, 9% moisture, and 2% ash. However, the chemical composition of yuba depends on the composition of the soymilk from which it is made, and on the stage at which the yuba film forms. In general, the protein and lipid contents of successively removed sheets decreases steadily, while the carbohydrate and ash contents increase (36).

Yuba is appreciated primarily for its unique flavor and texture, and is considered one of the oldest “texturized” protein foods. It is commonly used as a wrapper for other foods, or used in soups or cooked with other food materials. Due to limited production and high cost, yuba is considered a delicacy.

Okara. Okara, also known as soy pulp in English, and *doufu zha* or *dou zha* in Chinese, is the insoluble residue after filtration of soy slurry into soymilk. Therefore,

it is considered a by-product of soymilk and tofu preparation. Yet, for every pound of dry soybeans made into soymilk or tofu, about 1 lb of okara is generated. More specifically, on average, 53% of the initial soybean dry mass is recovered in tofu, 34% in okara, and 16% in whey. About 72% of soy protein is recovered in tofu, 23% in okara, and 8% in whey; the respective average percentages for soybean oil recovery are 82, 16, and <1 (37).

The major use of okara is as animal feed. However, there are various ways of using okara as food. For examples, in some parts of China, okara is salted and spiced and served as a pickle, or simply made into a dish with meat or vegetables. With growing awareness of the importance of dietary fiber for human health, there is an increasing interest in using okara as a food ingredient. Preparation through fermentation is an alternative method for value-added utilization of okara. An excellent review on okara is available in the literature (38).

Soybean Sprouts. Soybean sprouts are made by allowing soybeans to germinate under dark conditions. To produce soybean sprouts, soybeans—preferably freshly harvested, small- to medium-seeded beans with good vigor—are first soaked in warm water to full hydration, washed well, and then spread in thin layers in a deep container (or bucket) with holes at the bottom for water draining. The container is covered with hay or other material to screen out light but allow air exchange, and then placed where the temperature is kept at about 23°C. The beans in the container are sprinkled with water 3–4 times a day. Addition of water not only provides moisture for seeds to germinate and for new seedlings to grow but also helps to reduce heat built up due to active seed metabolism during germination. However, excessive moisture is unfavorable for rapid sprouting, as it tends to limit oxygen supply. Also, light should always be avoided during the process as it causes sprouts to develop roots and turn green, both of which are undesirable. In less than a week, when a majority of sprouts reach a length of about 8 cm, they are ready for harvesting, and are washed and dehulled.

The finished product is crispy, comprising yellowish cotyledons and a long, bright white sprout. It has a distinct taste, which may be described as beany by Westerners. In a typical germination process, 1 lb of dry soybeans can produce 7–9 lb of fresh bean sprouts. Commercial production of soy sprouts nowadays often uses automatic bean sprout growing systems, which may feature a computer system to control the water temperature and the watering schedule and overhead sprayers to provide an even distribution of a controlled amount of water. Furthermore, some systems can be set to add a nutrient, to wash the full-grown sprouts, and even to recycle the spray water. By use of such equipment, what used to be a painstaking task of growing soy sprouts now becomes fully automatic.

Compared with original dry soybeans, soy sprouts offer several nutritional advantages. First, germination causes significant increase in several vitamins, including ascorbic acid (vitamin C), riboflavin, and thiamine (39,40). Second, the flatulence-causing oligosaccharides, mainly stachyose and raffinose, are metabolized during sprouting (41). Third, phytic acid is also reduced due to increased phytase

activity (42). Fourth, germination causes increases in aspartic and glutamic acids, which contribute to nutrition and savory flavor of the final product (43). Furthermore, germination reduces beany flavor and improves organoleptic qualities of soybean seeds (40).

Soybean sprouts are very popular in Korea and southern China, serving as a vegetable throughout the year. They are used in soups, salads, and side dishes. During cooking, it is desirable to minimize heating to maintain the inherent crisp texture and distinct taste and to minimize destruction of vitamins. With the migration of Asians and their popular cuisine to new places, and with growing interest in soyfoods, the demand for soybean sprouts has grown worldwide.

Vegetable Soybeans. With a green or greenish-yellow color, soft texture, and large seed size (due to high moisture content and specially selected varieties), vegetable soybeans are normally picked at about 80% maturity in the greenish-yellow pod from the field. Therefore, they are also known as immature soybeans or fresh green soybeans (44).

Direct consumption of green vegetable soybeans is very popular in China, Japan, and some other Far East countries and regions. Steamed or boiled in water before or after shelling, normally for less than 20 min, and lightly salted or spiced, these immature beans can be served either as a delicious green vegetable with a main meal or as a tasty hors d'oeuvre, often with beer or other alcoholic drinks. In Japan, immature soybeans are known as *edamame*, and are sold fresh or frozen in the market. They may also be made into roasted beans, which have a crunchy texture and greenish-beige color, and sold as *Irori mame*.

Vegetable soybeans are highly nutritious. Compared with mature soybeans, they contain higher amounts of ascorbic acid and beta-carotene, and lower levels of trypsin inhibitors, oligosaccharides, and phytate, and ultimately have higher scores on the protein efficiency ratio scale in rats. When compared with other frozen vegetables, such as frozen peas and corn, green vegetable soybeans have higher levels of protein, oil, fiber, iron, and calcium (45).

In the West, green vegetable soybeans have gained much popularity in recent years, due to their high nutrition, tender texture, sweet and delicious taste, little beany flavor, and versatility for processing. The product is marketed mainly in the three different forms fresh, frozen, and canned; frozen immature soybeans are most popular. They can be used in side dishes, salads, tacos, rice dishes, casseroles, mixed vegetable dishes, soups, stews, stir-fry dishes, and meat dishes. They can be cooked over a stovetop, in a microwave, or in a steamer. Therefore, developing and marketing green vegetable soybeans would help expand food uses of soybeans and meet an increasing demand for soyfoods (46). Chapter 11 covers vegetable soybeans in detail.

Roasted (Soynuts) or Cooked Whole Soybeans. When clean, whole soybeans are roasted for about 30 min, they become brown and acquire a characteristic toasted flavor. Upon cooling, the roasted beans, known as soynuts, can be used, like roasted

peanuts, as a snack or ingredient to add a crunchy texture and nutlike flavor to a wide variety of salads, sauces, casseroles, and miso preparations. Besides dry-roasting, whole soybeans may be oil-roasted.

When roasted soybeans are ground into powder, they become roasted soy powder, which is similar to modern full-fat soy flour except that it contains the seed coat and has a nutty flavor. The product is known as *doufen* in Chinese and *kinako* in Japanese. Roasted soy flour can be used as a filling or topping, for example, as a spread on rice or rice cakes.

Whole soybeans can also be consumed directly after soaking and cooking (steaming or boiling) until their texture becomes tender. Salt, oil, soy sauce, and other spices and seasonings may be added during cooking.

Fermented Soyfoods

There are four major fermented soyfoods (soy paste, soy sauce, tempeh, and natto) and three minor fermented soyfoods (sufu, soy nuggets, and soy yogurts). Fermented soyfoods vary greatly in the microorganisms involved, methods of preparation, length of fermentation, principles of processing, and end uses. While it takes only a few days to prepare tempeh and natto, preparation of the remaining types of fermented soyfoods generally requires several months to complete. Except for natto and soy yogurts, which result from bacterial fermentation, all others are fermented mainly through fungal fermentation. A few products, such as fermented soy paste, soy sauce, and soy nuggets even share the same type of microorganisms, *Aspergillus* sp. In terms of end uses, most fermented soyfoods, including soy paste, soy sauce, soy nuggets, and sufu, are generally used as seasonings in cooking or making soups. They contribute more in flavor than in nutrition to the diet. They are characterized by high salt content because salt is added during the second stage of fermentation, as well as by the presence of certain by-products (such as acids and alcohols) from desirable fermentation. Both salt and by-products inhibit or slow spoilage of these products and allow them to have a relatively long shelf life. The remaining types, including tempeh, natto, and soy yogurts, contain no added salt, and are consumed as part of the main meal. Thus they contribute protein and oil to the diet as well as their characteristic flavor. For recent reviews on fermented soyfoods, see Shi and Ren (47), Liu (15), and Hui *et al.* (16).

Fermented Soy Paste (Jiang and Miso). Soy paste is an important fermented soyfood in the Far East. It has a color varying from a light, bright yellow to a nearly black brown, a distinctively pleasant aroma, and a salty taste. Soy paste is commonly known as *jiang* (Mandarin) or *chiang* (Cantonese) in China; *miso* in Japan; *jang* in Korea; *taucho* in Indonesia; and *taotsi* in the Philippines.

Developed in China some 2,500 years ago, *jiang* was the progenitor of the many varieties of soy paste and soy sauce that are now used throughout the world. At present, Chinese *jiang* and Japanese *miso* are the two most popular types of soy paste. Although sharing the same progenitor and same microorganisms, *Aspergillus oryzae*

and/or *A. sojae*, the two differ in many aspects. Chinese jiang is made from soybeans and wheat flour. The finished product may be unground so that individual particles of soybeans are present. It is used mainly as an all-purpose seasoning for dishes and soups. However, Japanese miso is made from soybeans mixed with rice or barley, or from soybeans alone. The finished product is a paste resembling peanut butter in consistency and may have a sweet taste. It is mainly dissolved in water as a base for various types of soups in Japan.

The method for making miso may vary with soybean variety, but the basic process is essentially the same as that for making Chinese jiang. For example, Japanese rice miso is made in five distinct steps: preparation of rice koji, treatment of soybeans, mixing and mashing of all ingredients, fermentation, and pasteurization and packaging. For details see Shurtleff and Aoyagi (48), Liu (15), and Hui *et al.* (16); the following is an outline of the steps:

1. *Preparing rice koji.* Non-glutinous, polished rice is cleaned, washed, and soaked overnight and then steamed for about 40 min. When cooled to 35°C, the cooked rice is inoculated with koji starter containing *A. oryzae* spores. This is followed by incubation at 30–35°C and a relative humidity higher than 90%. After about 40 h of inoculation, when the cooked rice is completely covered with white mycelium, it becomes a fermented mass known as koji.
2. *Treating soybeans.* Concurrent with the koji preparation, the whole soybeans are cleaned, washed, and soaked in water overnight. They are then cooked in boiling water.
3. *Mixing and mashing.* After cooling to room temperature, the cooked soybeans are mixed with salted rice koji and water containing inoculum, which may come from a previous batch or pure culture. The mixed materials are roughly mashed by passing them through a motor-driven chopper with 5-mm perforations.
4. *Fermenting.* After mixing and mashing, the mixture is packed tightly into open tanks or vats. The young miso is allowed to ferment at a controlled temperature, normally in the range of 30–38°C for a period up to 6 months, depending on the type of miso to be made.
5. *Pasteurizing and packaging.* After ripening, miso is blended if necessary, and mashed again through a chopper with a plate cutter having perforations of 1–2 mm. The mashed miso is then packaged in a resin bag or cubic container for markets after being pasteurized with a steam jacket or mixing with preservatives such as 2% ethyl alcohol or 0.1% sorbic acid.

Soy Sauce. Soy sauce is a dark-brown liquid extracted from a fermented mixture of soybeans and wheat. It is known as *jiangyou* in Chinese and *shuyu* in Japanese. With a salty taste and sharp flavor, soy sauce has been served as an all-purpose seasoning for thousands of years.

Today, among all the soyfoods, soy sauce is the most widely accepted product in Western countries. This is because as an all-purpose seasoning, soy sauce offers a

wide range of applications. Soy sauce not only contributes a unique flavor profile to traditional Asian foods but also holds great potential as a flavoring and flavor-enhancing material for a wide variety of non-Asian food products. Furthermore, the acids, alcohols, and salts present in soy sauce contribute to the overall preservative effect as well as antioxidant effect (49), and many amino acids have been identified both as flavor potentiators and umami contributors, most notably glutamic acid. Therefore, besides contributing directly to flavor, soy sauce contributes functional benefits to processed food and also serves as a natural flavor enhancer.

The principle and general steps of soy sauce making are similar to those of miso making. The basic steps include treatment of raw materials, koji making, brine fermentation, pressing, and refining. Soy sauce is covered in detail in Chapter 13.

Japanese Natto. Originating in the northern part of Japan about 1,000 years ago, natto is one of the few products in which bacteria predominate during fermentation. When properly prepared, it has a slimy appearance, sweet taste, and a characteristic aroma (Fig. 2.7). In Japan, natto is often eaten with soy sauce or mustard, and served for breakfast and dinner along with rice.

To make natto, soybeans, preferably small-seeded, are washed and soaked in water overnight (Fig. 2.8). The soaked beans are then cooked in a steamer or a pressure cooker for about 30 min, or until the beans are soft. Cooked beans are then drained and cooled to about 40°C. The cooked beans are then inoculated with a pure-culture suspension of *Bacillus natto* and thoroughly mixed before being packed in wooden boxes or polyethylene bags. The polyethylene bags are perforated from the outset for good aeration. The packages are put into shallow sliced-wood or polystyrene trays and set in a warm, thermostatic chamber with the controlled temperature at 40°C. After 14–20 h of fermentation, the bacteria will have covered the beans with a white sticky coating, indicating the time for harvesting. For better quality, the package may be kept at a refrigerating temperature for 1–2 d to allow maturation and then taken out for consumption or retailing as needed.

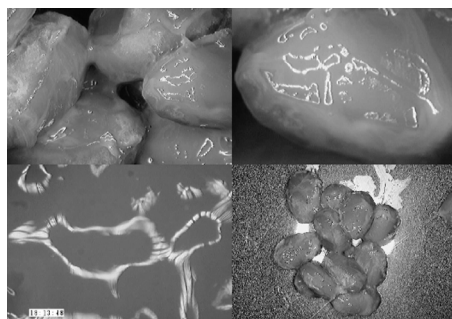


Figure 2.7. Natto, a fermented Japanese soyfood.

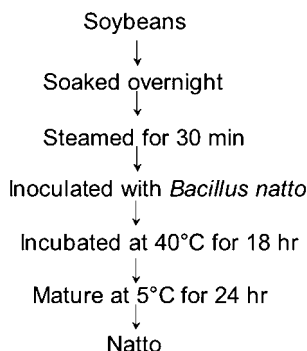


Figure 2.8. Natto production outline.

Unlike preparations of many other fermented soyfoods, which are complex and require actions of multiple microorganisms with a mold dominating, preparation of natto is relatively simple and requires action of only one type of microorganism—*Bacillus natto* (50). During fermentation, *B. natto* bacteria grow, multiply, and sporulate. One of the most remarkable features of the genus *Bacillus* is the secretion of various extracellular enzymes, including protease, amylase, gamma-glutamyltranspeptidase (GTP), levansucrase, and phytase. As natto bacilli grow, the enzymes they secrete or produce catalyze many chemical reactions that lead to production of the characteristic sticky material as well as to formation of the characteristic aroma and flavor of natto. The viscous material consists of polysaccharide (a levan-form fructan) and gamma-polyglutamic acid (51).

Recent research has shown the health benefits of natto. In particular, natto has been shown to contain significant amount of vitamin K₂, which is derived from the microorganism *Bacillus subtilis* (natto). Vitamin K₂ is the cofactor that converts nonactivated osteocalcin into activated osteocalcin by carboxylation. In rat studies as well as in vitro, natto has been shown to promote formation of osteocalcin, a bone protein, and to participate in bone formation (52,53).

Tempeh. Tempeh, or tempe in some literature, is made by fermenting dehulled and cooked soybeans with mold, *Rhizopus* sp. Freshly prepared tempeh is a cake-like product, covered and penetrated completely by white mycelium, and has a clean, yeasty odor. When sliced then deep-fat fried, it has a nutty flavor, pleasant aroma, and crunchy texture, often serving as a main dish or meat substitute.

Tempeh is widely believed to originate in Indonesia centuries ago. Tempeh continues to be one of the most popular fermented foods in Indonesia. Because of its meat-like texture and mushroom flavor, tempeh is well suited to Western tastes. It is becoming a popular food for a number of vegetarians in the United States and other parts of the world.

Traditionally, making tempeh is a household art in Indonesia. Soybeans are cleaned and then boiled in water for 30 min before hand dehulling. The dehulled beans are soaked overnight to allow full hydration and lactic acid fermentation and then cooked again for 60 min before inoculation with a starter containing *R. oligosporus* spores. The mixture is wrapped in banana leaves or perforated plastic bags, approximately a quarter pound per package. Fermentation is allowed to occur at room temperature for up to 18 h, or until the beans are bound by white mycelium. Alternatively, inoculated beans are spread on shallow aluminum foil or metal trays with perforated bottoms and covered with layers of banana leaves, waxed paper, or plastic films that are also perforated. Detailed discussion on tempeh is treated in Chapter 12.

Sufu or Chinese Cheese. When fresh tofu is fermented with a strain of certain fungi such as *Mucor hiemalis* or *Actinomucor elegans*, it becomes a new product known as sufu or Chinese cheese. The product, known as *doufu ru* or *furu* in mandarin Chinese, consists of tofu cubes covered with white or yellowish-white fungous mycelia, having a creamy, cheese-like consistency, salty taste, and characteristic flavor. It has a long history and written records date back to the Wei Dynasty (220–265 AD) in China. Today, sufu is still a popular dish consumed mainly with breakfast rice or steamed bread by all segments of the Chinese people, including those living overseas.

There are several types of sufu in the market, based on processing methods or color and flavor. Different choices of processing methods can result in mold-fermented sufu, naturally fermented sufu, bacteria-fermented sufu, or enzymatically ripened sufu, while choice of dressing mixture can produce red, white, or gray sufu. Flavorings commonly used include sugar, wine, chilies, soy sauce, sesame oil, rose essence, and others. More information on sufu can be found in Shi and Ren (47) and in Teng *et al.* (54).

Soy Nuggets (Douchi or Hamanatto). Soy nuggets, known as *douchi* in Mandarin Chinese and *hamanatto* in Japanese, are made by fermenting whole soybeans with strains of *Aspergillus oryzae*, although some other strains of fungi or bacteria may also be responsible. The finished product consists of intact beans with blackish color, and has a salty taste and a flavor similar to *jiang* or soy sauce. Because of its black color it is also known as salted black beans in the West. Soy nuggets are commonly used as an appetizer to be consumed with bland food, or as a flavoring agent to be cooked with vegetables, meats, and seafoods.

Originating in China before the Han dynasty (206 BC), the soy nugget is considered to be the progenitor of many types of fermented soy paste and soy sauce. It is the first soyfood to be described in written records. The preparation method, principles, and microorganisms involved in making soy nuggets are similar to those of fermented soy paste or soy sauce. Because of relatively high salt and low water contents, the product can be kept for a long time (47,55).

Soy Protein Products

In modern processing, soybeans are cracked to remove the hull and rolled into full-fat flakes for solvent extraction. After the oil has been extracted, the solvent is removed, and the flakes are dried, resulting in defatted soy flakes.

Soy protein products are mostly made from these defatted soy flakes. They are not consumed directly as food but instead find wide application as a versatile ingredient in virtually every type of food system, including bakery, dairy, meat, breakfast cereal, beverages, infant formula, and dairy and meat analogs. In these food systems, they not only boost protein content but also provide many functional properties, including gelling, emulsifying, water-holding, and fat-absorbing properties (56). There are four major types of soy protein products: flour, concentrates, isolates, and textured soy protein (Fig. 2.9).

Soy Flour

Soy flour is one of the least-processed soy protein products. It comes in many types, including full-fat, low-fat, and defatted; there are enzyme-active, toasted, and textured varieties of each of these. Defatted soy flour has been the most common type. It is produced by grinding defatted soy flakes and has a protein content of about 50%. It is mainly used as an ingredient in the bakery industry (57). However, full-fat soy flour has been gaining popularity in recent years (58). Low-fat soy flour can be made by expelling oil from soybeans then milling the meal (59). Detailed coverage on soy flour products is treated in Chapters 5 and 9.

Soy Protein Concentrate

Soy protein concentrate is traditionally made by aqueous alcohol extraction of defatted soy flakes. The resulting product has about 70% protein, with the remaining portion being mainly insoluble carbohydrates. The product may be further processed by thermal processing and homogenization for better functionality. Alternatively, soy concentrate can be made by an acid-leach method to retain isoflavones and other

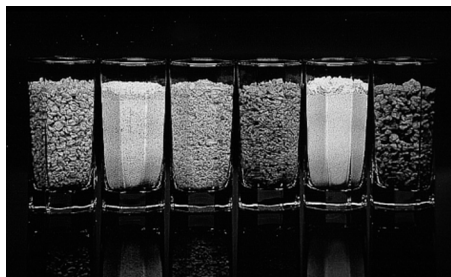


Figure 2.9. Soy protein products.

beneficial phytochemicals and to prevent protein denaturation. A versatile ingredient, soy protein concentrate is widely used in the meat industry to bind water and emulsify fat, and as a key ingredient of many meat alternatives. It is also used for protein fortification of various types of food. Detailed coverage of soy concentrate and the by-product of its processing—soy molasses—is presented in Chapters 6 and 10, respectively.

Soy Protein Isolate

Soy protein isolate is produced by alkaline extraction followed by precipitation at acid pH. As a result, both soluble and insoluble carbohydrates are removed. The resulting product has a protein content of 90%, and is light in color and bland in flavor. Soy isolate is the most-refined soy protein product, possessing many functional properties, including gelation and emulsification. As a result, it may be used in a wide range of food applications, including processed meat, meat analogs, soup and sauce bases, nutritional beverages, infant formulas, and dairy replacements. Chapter 7 provides detailed information about the product.

Textured Soy Proteins

Protein texturization is a process to impart a structure, like that of fiber, to a proteinaceous material. The resulting product is textured protein (60), which is further defined as food products made from edible protein sources. Textured protein products are characterized by having structural integrity and identifiable texture, which would enable them to withstand hydration in cooking and other preparations. Thus, texturization into fibrous meat analogs has been a unique way to make vegetable proteins palatable.

For the past several decades, many different processes have been developed and used to texturize soy proteins, each based on different starting materials. These include fiber spinning, thermoplastic extrusion, direct steam texturization, shaping and heating, enzymatic texturization, and high-moisture extrusion. The starting material may be defatted soy flour, concentrate, isolate, or a blend of several proteinaceous products (61–63).

Among all the approaches, for many years, thermoplastic extrusion has been the method of choice for soy protein texturization. In a typical thermoplastic extrusion process, dry proteinaceous materials, predominantly defatted soy flour or soy concentrate, are mixed with water, salts, and flavorings (for flavor and odor control), and then fed into a single-screw extruder. Under a high-temperature and low-moisture (<30%) condition, the product expands rapidly upon emerging from the die. The products are formed in a variety of shapes, sizes, and colors. The most popular shapes are granules, chunks, and flakes. Their uses have ranged from meat extenders to meat analogs, although the market for meat extenders has been far more successful. When used for meat analogs, textured proteins are frequently flavored and formulated to resemble meat, poultry, or seafood, which they may replace both in structure and appearance. Textured protein must be rehydrated with water before

use. Because of the spongy structure due to expansion, these products have poor flavor retention and lack real fibrous texture.

Recent development in extrusion technology has focused on using twin-screw extruders under high-moisture (40–80%) conditions for texturizing vegetable proteins into fibrous meat alternatives (64–66). In the high-moisture twin-screw process, the raw materials, predominantly soy protein, are mixed and fed to a twin-screw extruder, where a proper amount of water is added in and all ingredients are further blended and then melted by the thermomechanical action of the screws. The low velocity of the product through the die and the cooling of the product help create long strands of textured proteins. The resulting products resemble chicken or turkey breast meat (Fig. 2.10) and have enhanced visual appearance and taste sensation, and thus this process shows a great deal of promise for becoming a prominent method of texturizing vegetable proteins to meet increasing consumer demands for healthy and tasty foods. Already, several large protein ingredient companies in North America have invested in this technology and new high-moisture extruded products have entered the market in 2004.

Modern Soyfoods

In the West, many traditional soyfoods have been modified to suit local tastes (Fig. 2.11). These modified soyfoods, together with foods made mainly from soy protein ingredients by modern technology are known collectively as the new generation of soyfoods or modern soyfoods. They may look like and even taste like Western foods. The common features of this type of soyfoods include (a) they are soy-based products with soy as a main ingredient derived either from traditional soyfoods such as soymilk or tofu, or from modern soy ingredients such as soy protein concentrate or isolate, or a combination; (b) they are made through modern processing technology or a blending of traditional and modern methods,



Figure 2.10. Meat analog made by high-moisture extrusion of soybean protein.



Figure 2.11. New generation of soyfoods in the market.

and (c) they suit local or regional tastes and may resemble certain local foods in appearance, texture, or possibly taste.

Soy-based meat and dairy alternatives are two major subgroups of this category. Examples include soy ice cream, soy yogurts, soy cheese, soyburgers, meatless meatballs, imitation bacon bits, soy butter, soy puddings, tofu spreads and dressings—you name it. Detailed discussion of this category can be found in Liu (15).

Soy-Enriched Foods

One way to increase soy consumption is to incorporate soy into mainstream foods that Westerners or local people already eat and are familiar with. The idea is not new, but it differs from past practices in the amount of soy added. The new trend is to enrich common foods with a sufficient amount of soy protein so that consumers have the chance to eat several servings per day to reap the health benefits of soy. Among these new applications are soy bread, soy pastes, soy cereals, soy snacks, and so on (Fig. 2.12). The difference between soy-enriched foods and modern soyfoods lies in the fact that soy is the main ingredient in the latter.

Since a wide variety of products can be enriched with soy ingredients at varying levels, this category represents a large and growing category, providing multitudinous ways for consumers to incorporate soy into their diet. This trend is being accelerated recently due to the popularity of low-carbohydrate diets for weight control. Although the efficiency and scientific principle of a low-carbohydrate diet in reducing body weight is still controversial, and sustainability of such trend is questionable (we can still remember the rise and fall of low-fat foods in the 1990s), the food industry is busy developing new product lines that are low in carbohydrates and high in protein in order to capture the profit of this new fad. One of the ideal choices for increasing protein contents is to enrich food products with soy protein products.



Figure 2.12. Soy-enriched bakery products.
Courtesy of Cargill, Inc.

Functional Soy Ingredients/Dietary Supplements

As discussed in Chapter 1, soybeans are a powerhouse of phytochemicals. Among them are lecithin, isoflavones, oligosaccharides, tocopherols, sterols, phytates, and trypsin inhibitors. Generally speaking, most of these substances are associated with by-products of modern soybean processing. Some soy processors have made efforts to recover some of these substances and make them commercially available as ingredients for functional foods or dietary supplements. They represent yet another new type of soybean food use.

Soy Lecithin

Commercially, the term “lecithin” refers to a wide variety of products that have phosphatides as the sole or major components. Soy lecithin refers to a group of phospholipids naturally present in soybeans (1–3%), mainly phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid. Crude soy lecithin is a by-product produced during degumming of soybean oil. It is then dried, de-oiled by acetone, and may be subsequently chemically modified. Soy lecithin has many functional properties, including emulsifying, wetting, colloidal, and antioxidant properties. It also exerts some physiological effects on humans and animals. Therefore, it has multiple uses, such as in food, beverages, animal feed, health and nutritional products, cosmetics, and industrial coatings. For the majority of these uses, relatively small amounts of the lecithin are needed, often at a level of 0.1 to 2%. At such low levels, the color, flavor, and odor of the lecithin normally are not noticeable.

For edible applications, soy lecithin is normally added to such food products as shortening, margarine, baked goods, chocolate, confectionery coatings, peanut butter, powder mixes, and dietary food. In most cases, lecithin functions as a useful emulsifier. For example, when added to margarine, the lecithin prevents “sweeping” or “bleeding” of the moisture present, reduces spattering during frying, increases the

shortening effect for baking applications, and helps protect the vitamin A in fortified margarine from oxidation. When shortenings are formulated with lecithin, they become emulsified and widely used in baked goods, such as bread, biscuits, crackers, and cakes. Lecithin helps bring about rapid and intimate mixing of the shortening in the dough, improves the fermentation, water absorption, and handling characteristics of the dough, gives a more tender and richer product after baking, and prevents baked goods from going stale. Literature covering the subject includes Erickson (17) Sipos and Szuhaj (67).

Oligosaccharides

Oligosaccharides in mature soybeans are mainly raffinose (0.1–0.9%) and stachyose (1.4–4.1%) (68). Raffinose contains a fructose, a glucose, and a galactose, while stachyose contains an additional galactose. Both have a beta-fructosidic linkage and an alpha-galactosidic linkage. Their presence in soybeans has been linked with flatulence associated with human consumption of soy products, and therefore is generally considered undesirable. Yet, according to Tomomatsu (69), oligosaccharides are a powerful prebiotic and have been successfully commercialized in Japan for years. A prebiotic is defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. It is a substance that modifies the composition of the colonic microflora in such a way that a few of the potentially health-promoting bacteria, especially lactobacilli and bifidobacteria, become predominant in numbers (70).

Isoflavones

The soybean is unique in that it contains abundant isoflavones (1–4 mg/g dry matter), whereas most other types of food materials do not contain them (23,32). The isoflavones in soybeans are of three primary types, with each type being present in four chemical forms. Therefore, there are 12 isomers. Daidzein, genistein, and glycitein are aglucones. When glucosided, they become daidzin, genistin, and glycitin, respectively. In various experimental models, isoflavones have been shown to inhibit the growth of cancer cells, lower cholesterol levels, and inhibit bone resorption (5,8,71). These attributes are clearly relevant to chronic disease prevention and treatment. In addition, there is a relationship between soy consumption and relief of menopausal symptoms in certain women. It is hypothesized that soy isoflavones can act as estrogen agonists in the low-estrogen makeup of postmenopausal women, since both have similar chemical structures (4,5).

Concentrated and purified soy isoflavones are now commercially available in various forms (Fig. 2.13). They are produced mostly by patented procedures, from three main sources: soy molasses, soy germ, and defatted soy flakes. Chapter 3 covers isoflavones with respect to chemistry, occurrence, processing effects, health benefits, and commercial production by different procedures, and Chapter 9 discusses soy molasses and recovery of isoflavones from them.



Figure 2.13. Concentrated soy isoflavone product. Courtesy of Archer Daniels Midland Co.

Tocopherols

Tocopherol is known as vitamin E. It has four isomers, namely, alpha-, beta-, gamma-, and delta-tocopherol. The amount of alpha-, gamma-, and delta-tocopherols in the soybean range from 10.9–28.4, 150.0–191.0, and 24.6–72.5 $\mu\text{g/g}$ (72). During solvent extraction of soybeans, tocopherol goes with the oil fraction. It is lost mainly in the deodorization step of oil refinement, although the lost part can be recovered in commercial quantity.

Phytosterols

Phytosterols comprise a number of compounds structurally related to cholesterol. At least 44 phytosterols have been identified in plants, but only three major ones, beta-sitosterol, campesterol, and stigmasterol, are found in soybeans. Phytosterols are known to have cholesterol-lowering properties and possibly the ability to reduce cancer risk (73,74). A margarine containing beta-sitosterol or other sterols or stanols has become commercially available in recent years.

Trypsin Inhibitors

Trypsin inhibitors present in soybeans are of two primary types: Kunitz inhibitor and Bowman-Birk inhibitor (BBI). They are proteins in nature. By binding to the digestive enzyme trypsin, soy trypsin inhibitors adversely affect growth and in some animal models can cause pancreatic hypertrophy (75). On the other hand, much research has demonstrated the anticarcinogenic activity of BBI (3). Therefore, like some other phytochemicals, the nutritional significance and health benefits of soybean proteinase inhibitors for humans continue to be a debatable subject. Kennedy and Szuhaj (76) received a U.S. patent for making a Bowman-Birk inhibitor concentration for treatment of premalignant tissues.

In conclusion, although soybean production and utilization as food arose in ancient China several thousands of years ago, only recently are we rediscovering the value of this ancient bean for its functional health benefits and its potential to suit Westerners' tastes in various forms of food. For the general population to reap the health benefits of soy, one major challenge has been to incorporate soy into our diets. Although some phytochemicals in soybeans can be made into pills, there is no better way to benefit from soy than consuming soybeans as food on a regular basis. Fortunately, due to advancements in food technology, plant breeding, and human creativity, soybeans have been made into various types of foods and ingredients. Many traditional soyfoods have been modernized. Thousands of new products have been put into the market. Still, many are yet to come as corporate investment in research and development expands and consumers' interest in eating soy intensifies.

References

1. Golbitz, P., Soyfoods Sales Reach \$4.0 Billion in U.S. Bluebook Update, *Soyatech Publication 11*(April–June):1,9 (2004).
2. Anderson, J.W., B.M. Johnstone, and M.E. Cook-Newell, Meta-analysis of the Effects of Soy Protein Intake on Serum Lipids, *New Engl. J. Med.* 333:276–282 (1995).
3. Kennedy, A.R., The Evidence of Soybean Products as Cancer Preventive Agents, *J. Nutr.* 125:733S (1995).
4. Barnes, S., Evolution of the Health Benefits of Soy Isoflavones, *Proc. Soc. Exp. Biol. Med.* 217:386–392 (1998).
5. Setchell, K.D.R., and A. Cassidy, Dietary Isoflavones: Biological Effects and Relevance to Human Health, *J. Nutr.* 129:758S–767S (1999).
6. Friedman, M., and D.L. Brandon, Nutritional and Health Benefits of Soy Proteins, *J. Agric. Food Chem.* 49:1069–1086 (2001).
7. Messina, M., Soyfoods: Their Role in Disease Prevention and Treatment, in *Soybeans: Chemistry, Technology, and Utilization*, edited by K.S. Liu, Aspen Publishers, Gaithersburg, Maryland, 1999, pp. 442–477.
8. Messina, M., Legumes and Soybeans: Overview of Their Nutritional Profiles and Health Effects, *Am. J. Clin. Nutr.* 70:439S–450S (1999).
9. Erdman, J.W., Soy Protein and Cardiovascular Disease: A Statement for Healthcare Professionals from the Nutrition Committee of AHA, *Circulation* 102:2555–2559 (2000).
10. Saio, K., Current Developments in Soyfood Processing in East Asia, in *Proceedings of Invited and Contributed Papers and Posters for World Soybean Research Conference VI*, edited by H.E. Kauffman, University of Illinois, Urbana-Champaign, 1999, pp. 372–379.
11. Liu, K., Expanding Soybean Food Utilization, *Food Technol.* 54:46–58 (2000).
12. Liu, K.S., Blending Modern and Traditional Processing to Create the Next Breakthrough, presented at Soyfoods Summit 2004, San Diego, California, February 18–20, 2004.
13. Shurtleff, W., and A. Aoyagi, *Tofu and Soymilk Production*, The Soyfoods Center, Lafayette, California, 1984.
14. Wang, X.L., *et al.*, *Chinese Soybean Products* [in Chinese], China Light Industry Publisher, Beijing, China, 1997.
15. Liu, K.S., *Soybeans: Chemistry, Technology, and Utilization*, Aspen Publishers, Gaithersburg, Maryland, 1999.

16. Hui, Y.H., L. Meunier-Goddik, A.S. Hansen, W-K Nip, P.S. Stanfield, and F. Toldra (Eds.), *Handbook of Food and Beverage Fermentation Technology*, Marcel Dekker, New York, 2004.
17. Erickson, D.R. (Ed.), *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995.
18. Hui, Y.H. (Ed.), *Bailey's Industrial Oil and Fat Products Vol. 3. Edible Oil and Fat Products: Products and Application Technology* (5th ed.), John Wiley & Sons, New York, 1996.
19. Liu, K.S., Modifying Soybean Oil through Plant Breeding and Genetic Engineering, in *World Oilseed Conference Proceedings*, edited by R.L Wilson, AOCS Press, Champaign, Illinois, 2001, pp. 84–89.
20. Fehr, W.R., and C.F. Curtiss, Breeding for Fatty Acid Composition of Soybean Oil, in *Proceedings, VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 815–821.
21. Kwok, K.C., and K. Niranjan, Effect of Thermal Processing on Soymilk, *Intl. J. Food Sci. Technol.* 30:263–265 (1995).
22. Golbitz, P., The Use of Whole Soybeans as Food Ingredients, presented at Soyfoods Summit 2003, Miami, Florida, February 26–28, 2003.
23. Coward, L., N.C. Barnes, K.D.R. Setchell, and S. Barnes, Genistein, Daidzein, and Their beta-Glycoside Conjugates: Antitumor Isoflavones in Soybean Foods from American and Asian Diets, *J. Agric. Food Chem.* 41:1961–1967 (1993).
24. Coward, L., M. Smith, M. Kirk, and S. Barnes, Chemical Modification of Isoflavones in Soyfoods during Cooking and Processing, *Am. J. Clin. Nutr.* 68:1486S–1491S (1998).
25. Wang, H.-J., and P.A. Murphy, Isoflavone Composition of American and Japanese Soybeans in Iowa: Effects of Variety, Crop Year and Location, *J. Agric. Food Chem.* 42:1674–1677 (1994).
26. Chen, S., Preparation of Fluid Soymilk, in *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, AOCS Press, Champaign, Illinois, 1989, pp. 341–351.
27. Imram, N. (Ed.), *Soya Handbook*, Tetra Pak, Singapore, 2003.
28. Liu, K.S., Tofu and Prepared Tofu Products: Varieties and Processing, presented at Soyfoods Summit 2003, Miami, Florida, February 26–28, 2003.
29. Wang, H.L., E.W. Swain, and W.F. Kwolek, Effect of Soybean Varieties on the Yield and Quality of Tofu, *Cereal Chem.* 60:245 (1983).
30. Food and Drug Administration, Food Labeling: Health Claims, Soy Protein and Coronary Heart Disease, Department of Health and Human Services, Food and Drug Administration, 21 CFR Part 101, Oct. 26, 1999.
31. Jackson, C.J.C., J.P. Dini, C. Lavandier, H.P.V. Rupasinghe, H. Faulkner, V. Poysa, D. Buzzell, and S. DeGrandis, Effects of Processing on the Content and Composition of Isoflavones during Manufacturing of Soy Beverage and Tofu, *Process Biochem.* 37:1117–1123 (2002).
32. Wang, H.-J., and P.A. Murphy, Isoflavone Content in Commercial Soybean Foods, *J. Agric. Food Chem.* 42:1666–1673 (1994).
33. Wakai, K., I. Egami, K. Kato, T. Kawamura, A. Tamakoshi, Y. Lin, T. Nakayama, M. Wa, and Y. Ohno, Dietary Intake and Sources of Isoflavones among Japanese, *Nutr. Cancer* 33:139–145 (1999).

34. Shu, X.O., F. Jin, Q. Dai, W.Q. Wen, J.D. Potter, L.H. Kushi, Z.X. Ruan, Y.T. Gao, and W. Zheng, Soyfood Intake during Adolescence and Subsequent Risk of Breast Cancer among Chinese Women, *Cancer Epidemiol. Biom. Prev.* 10:483–488 (2001).
35. Brzezinski, A., *et al.*, Tofu Consumption Also Helps Alleviate Hot Flashes in Menopausal Women, *J. N. Am. Menopause Soc.* 4:89–94 (1997).
36. Okamoto, S., Factors Affecting Protein Film Formation, *Cereal Foods World* 23:256 (1978).
37. van der Riet, W.B., A.W. Wight, J.J. Cilliers, and J.M. Datel, Food Chemical Investigation of Tofu and Its Byproduct Okara, *Food Chem.* 34:193–202 (1989).
38. O'Toole, D.K., Characteristics and Use of Okara: The Soybean Residue from Soy Milk Production—A Review, *J. Agric. Food Chem.* 47:363–371 (1999).
39. Bates, R.P., and R.F. Matthews, Ascorbic Acid and β -carotene in Soybeans as Influenced by Maturity, Sprouting, Processing and Storage, *Proc. Fla. State Hort. Soc.* 88:266–271 (1975).
40. Ahmad, S., and D.K. Pathak, Nutritional Changes in Soybean during Germination, *J. Food Sci. Technol.* 37:665–666 (2000).
41. East, J.W., T.O.M. Nakayama, and S.B. Parkman, Changes in Stachyose, Raffinose, Sucrose and Monosaccharides during Germination of Soybeans, *Crop Sci.* 12:7 (1972).
42. Chen, H., and S.H. Pan, Decrease of Phytate during Germination of Pea Seeds, *Nutr. Rep. Intl.* 16:125–131 (1977).
43. Zhuang, B., and B. Xu, Changes of Protein and Its Composition, Fat and Its Composition in Different Species Seeds of Subgenus *soja* during Germination, in *Proceedings of World Soybean Research Conference IV*, Buenos Aires, Argentina, March 5–9, 1989, pp. 1019–1023.
44. Young, G., T. Mebrahtu, and J. Johnson, Acceptability of Green Soybeans as a Vegetable Entity, *Plant Foods for Human Nutr.* 55:323–333 (2000).
45. Liu, K.S., Immature Soybeans: Direct Use for Food, *INFORM* 7:1217–1223 (1996).
46. Shanmugasundaram, S., and M.R. Yan, Global Expansion of High Value Vegetable Soybean. Physiologically Active Peptides in Soybean and Soy Products, in *Proceedings, VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 915–920.
47. Shi, Y.G., and L. Ren (Ed.), *Soyfood Technology*, China's Light Industry Publisher, Beijing, China, 1993.
48. Shurtleff, W., and A. Aoyagi, *The Book of Miso*, Ten Speed Press, Berkeley, California, 1983.
49. Chiou, R.Y.Y., K.L. Ku, Y.S. Lai, and L.G. Chang, Antioxidative Characteristics of Oils in Ground Pork-Fat Patties Cooked with Soy Sauce, *J. Am. Oil Chem. Soc.* 78:7–11 (2001).
50. Muramatsu, K., Y. Kanai, N. Kimura, and K. Yoshida, Production of Natto with High Elastase Activity [in Japanese], *J. Jap. Soc. Food Sci. Technol.* 42:575–582 (1995).
51. Hara, T., Y. Fujio, and S. Ueda, Polyglutamate Production by *Bacillus subtilis* (Natto), *J. Appl. Biochem.* 2:112–120 (1982).
52. Yamaguchi, M., H. Taguchi, Y.H. Gao, A. Igarashi, and Y. Tsukamoto, Effect of Vitamin K₂ (Menaquinone-7) in Fermented Soybean (Natto) on Bone Loss in Ovariectomized Rats, *J. Bone Miner. Metab.* 17:23–29 (1999).

53. Yamaguchi, M., E. Sugimoto, S. Hachiya, and Y. Tsukamoto, Stimulatory Effect of Menaquinone-7 (Vitamin K₂) on Osteoclastic Bone Formation in Vitro, *Mol. Cell Biochem.* 223:131–137 (2001).
54. Teng, D.-F., C.-S. Lin, and P.-C. Hsieh, Fermented Tofu: Sufu and Stinky Tofu, in *Handbook of Food and Beverage Fermentation Technology*, edited by Y.H. Hui *et al.*, Marcel Dekker, New York, 2004, pp. 571–582.
55. Teng, D.-F., C.-S. Lin, and P.-C. Hsieh, Fermented Whole Soybeans and Soybean Paste, in *Handbook of Food and Beverage Fermentation Technology*, edited by Y.H. Hui *et al.*, Marcel Dekker, New York, 2004, pp. 533–570.
56. Egbert, R., Soy Protein in the Food Processing Industry, in *Proceedings of Invited and Contributed Papers and Posters for World Soybean Research Conference VI*, edited by H.E. Kauffman, University of Illinois, Urbana-Champaign, 1999, pp. 403–408.
57. Limpert, W.F., Soy Ingredients in Bakery and Other Cereal Products, in *Proceedings, VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 1152–1154.
58. Lang, P., Full-fat Soy Flour, Functionality and Applications, presented at Soyfoods 2001, Phoenix, Arizona, January 16–18, 2001.
59. Wijeratne, W.B., Alternative Technologies for Primary Processing of Soybean, in *Proceedings of Invited and Contributed Papers and Posters for World Soybean Research Conference VI*, edited by H.E. Kauffman, University of Illinois, Urbana-Champaign, 1999, pp. 368–370.
60. Lockmiller, N.R., Textured Protein Products, *Food Technol.* 26:56 (1972).
61. Kearns, J.P., G.J. Rokey, and G.R. Huber, Extrusion of Texturized Proteins, in *Proceedings of the World Congress: Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, AOCS Press, Champaign, Illinois, 1989, p. 353.
62. Areas, J.A.G., Extrusion of Food Proteins, *Crit. Rev. Food Sci. Nutr.* 31:365–392 (1992).
63. Shemer, M., G. Arbel, I. Bait-Halachmy, and Y. Arad, Fibrous Food Product and Method and Device for Its Production, U.S. Patent 6,319,539 B1, November 20, 2001.
64. Cheftel, J.C., M. Kitagawa, and C. Queguiner, New Protein Texturization Process by Extrusion Cooking at High Moisture Levels, *Food Rev. Intl.* 8:235–275 (1992).
65. Thiebaut, M., E. Dumay, and J.C. Cheftel, Influence of Process Variables on the Characteristics of High Moisture Fish Soy Protein Mix Texturized by Extrusion Cooking, *Lebensm.-Wiss. u-Technol.* 29:529–535 (1996).
66. Yao, G., K. Liu, and F. Hsieh, A New Method for Characterizing Fiber Formation in Meat Analogs during High Moisture Extrusion, *J. Food Sci.* in press, 2004.
67. Sipos, E.F., and B.F. Szuhaj, Lecithins, in *Bailey's Industrial Oil & Fat Products, Vol. I Edible Oil and Fat Products: General Applications*. 5th edn., edited by Y.H. Hui, John Wiley & Sons, New York, 1996, p. 311.
68. Hymowitz, T., F.I. Collins, J. Panczer, and W.M. Walker, Relationship between the Content of Oil, Protein, and Sugar in Soybean Seed, *Agron. J.* 64:613–616 (1972).
69. Tomomatsu, H., Health Effects of Oligosaccharides, *Food Technol.* 48:61–65 (1994).
70. Roberfroid, M.B., Prebiotics and Synbiotics: Concepts and Nutritional Properties, *Brit. J. Nutr.* 80:S197–S202 (1998).
71. Messina, M., Potential Public Health Implications of the Hypocholesterolemic Effects of Soy Protein, *Nutr.* 19:280–281 (2003).

72. Guzman, G.J., and P.A. Murphy, Tocopherols of Soybean Seeds and Soybean Curd (Tofu), *J. Agric. Food Chem.* 34:791–795 (1986).
73. Ling, W.H., and P.J.H. Jones, Dietary Phytosterols: A Review of Metabolism, Benefits and Side Effects, *Life Sci.* 57:195–206 (1995).
74. Phytosterols, *Crit. Rev. Food Sci. Nutr.* 39:275–283 (1999).
75. Liener, I.E., Implications of Antinutritional Components in Soybean Foods, *CRC Crit. Rev. Food Sci. Nutr.* 34:31–67 (1994).
76. Kennedy, A.R., and B.F. Szuhaj, Bowman-Birk Inhibitor Concentrate Compositions and Methods for the Treatment of Pre-malignant Tissue, U.S. Patent 5,505,946, April 9, 1996.

Chapter 3

Soy Isoflavones: Chemistry, Processing Effects, Health Benefits, and Commercial Production

KeShun Liu

University of Missouri, Columbia, MO 65211

For many years, soybeans have been primarily identified with their high oil and high protein content. However, during the past several years, there has been much interest among clinicians and researchers in the potential role of soyfoods in preventing and treating many chronic diseases. Increasing evidence has suggested that isoflavones in soybeans are the primary factor contributing to these health benefits (1–5). Consequently, there has been an upsurge in interest in isoflavones from soy and other plant sources. Isoflavones are a class of plant flavonoid compounds that have some weak estrogenic activity. Research has revealed many possible health benefits that may be achieved from the consumption of isoflavones, including lowering cholesterol levels, preventing prostate and breast cancer, preventing bone loss, and alleviating menopausal symptoms.

Coupled with this new development, in recent years a growing number of food and commodity processors have developed and aggressively marketed lines of concentrated soy isoflavone products that can be used as ingredients in food or beverages or incorporated into dietary supplements. Annual soy isoflavone sales in the United States are skyrocketing, with an annual growth rate of over 50% in recent years (6). The market for isoflavones in 2003 was estimated at \$500 million in the United States alone. The worldwide market for isoflavones is also expanding. There are several key contributing factors for this growing market for isoflavone products as food ingredients and dietary supplements, including a surge in consumer awareness and interest in natural solutions to health issues; scientific research that links soy isoflavones to many health benefits; low soyfood consumption and low natural levels of isoflavones in many soy food products, which make it difficult for consumers to meet the serving range needed to have a physiological impact; and low margins and slow growth in oilseed crushing operations.

This chapter provides information regarding soybean isoflavone chemical structure and occurrence, effects of food processing and assay methodology on isoflavone products, isoflavone content in various foods and supplements, the health benefits of isoflavones, and extraction and purification processes for research and commercial production.

Chemical Structure and Natural Occurrence

Isoflavones belong to a group of compounds that share a basic structure consisting of two benzyl rings joined by a three-carbon bridge, which may or may not be closed

in a pyran ring (Fig. 3.1). The structure is generally simplified as C₆-C₃-C₆. This group of compounds is known as flavonoids, which include by far the largest and most diverse range of plant phenolics. Besides isoflavones, other subclasses of flavonoids include red and blue anthocyanin pigments, flavones, flavonols, flavanols, aurones, and chalcones. Isoflavones differ from flavones in that the benzyl ring B is joined at position 3 instead of at position 2; compare the isoflavone structure shown in Figure 3.2 to the flavonoid skeleton shown in Figure 3.1. Isoflavones may be described as colorless, crystalline phenolic ketones, and their structures bear some similarity to estrogens, and thus possess weak estrogen activity.

Although flavonoids are found in various plant families in different tissues, isoflavones are present in just a few botanical families. This is because of the limited distribution of the enzyme chalcone isomerase that converts 2(*R*)-naringenin, a flavone precursor, into 2-hydroxydaidzein (7). The soybean is unique in that it contains the highest amount of isoflavones, normally in the range of 1–4 mg/g dry weight (8–12). Isoflavones are also found in a few other plant sources, including alfalfa, red clover, and kudzu root. Isoflavone concentration in flax and chickpeas is very low and likely nutritionally irrelevant.

The isoflavones in soybeans and soy products have three primary types: daidzein, genistein, and glycitein. Each of these three isomers, known as aglucones or free forms, can also exist in one glucoside form and two glucoside conjugate forms, acetylglucoside and malonylglucoside. Therefore, in total, there are 12 isomers of isoflavones in soybeans. In the β-glucoside form, the three aglucones become genistin, daidzin, and glycitin. In the acetylglucoside form, soybean isoflavones are named as 6''-*O*-acetyldaidzin, 6''-*O*-acetylgenistin, and 6''-*O*-acetylglycitin. In the malonylglucoside form, the corresponding names are 6''-*O*-malonyldaidzin, 6''-*O*-malonylgenistin, and 6''-*O*-malonylglycitin (Fig. 3.2).

The isoflavone content as well as distribution of isomers in soybeans is greatly influenced by many factors, including variety, growing locations, planting year, planting date, and harvesting date (13–16). For example, researchers at Iowa State University found that the total isoflavone content of a simple variety, Vinton 81, ranged from 0.84 to 1.64 mg/g raw seeds among eight locations in 1995, and from 1.61 to 2.84 mg/g in 1996 (12). In another Iowa study (13), a single variety grown

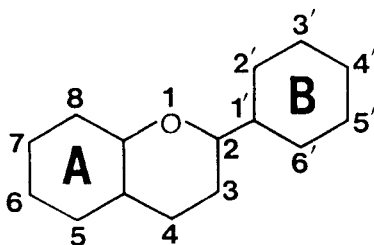
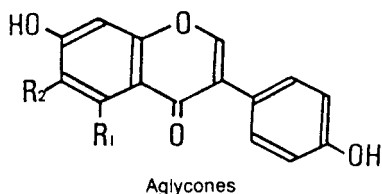
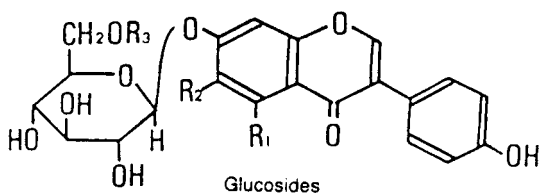


Figure 3.1. Flavonoid structural skeleton.



R ₁	R ₂	Compounds
H	H	daidzein
OH	H	genistein
H	OCH ₃	glycitein



R ₁	R ₂	R ₃	Compounds
H	H	H	daidzin
OH	H	H	genistin
H	OCH ₃	H	glycitin
H	H	COCH ₃	6''-O-Acetyldaidzin
OH	H	COCH ₃	6''-O-Acetylgenistin
H	OCH ₃	COCH ₃	6''-O-Acetylglycitin
H	H	COCH ₂ COOH	6''-O-Malonyldaidzin
OH	H	COCH ₂ COOH	6''-O-Malonylgenistin
H	OCH ₃	COCH ₂ COOH	6''-O-Malonyglycitin

Figure 3.2. Structures of the 12 soy isoflavones.

in different locations or crop years can have up to a five-fold difference in isoflavone concentration. The total isoflavone content in the tested soybean varieties ranged from 1.261 to 3.89 mg/g seed. Among the 12 isomers, 6''-O-malonylgenistin, genistin, 6''-O-malonyldaidzin, and daidzin are predominant. The distribution pattern of isomers differs between American and Japanese soybeans; Japanese soybeans have higher 6''-O-malonylglycitin contents and higher ratios of malonyldaidzin to daidzin and malonylgenistin to genistin. Similar findings are also observed when soybeans grown in Brazil (17) and Europe (14) are compared with soybeans grown in Japan (18). It appears that the environmental effect is much greater than genetics.

In addition, the concentration and composition of isoflavones vary greatly between structural parts within a soybean seed (10,18). The concentration of the total isoflavones in soybean hypocotyl is 5.5–6 times higher than that in cotyledons. Glycitein and its three derivatives occur exclusively in the hypocotyl. Seed coats are almost absent of isoflavones. Although the hypocotyl has a higher concentration of isoflavones, 80–90% of the total seed isoflavones are located in cotyledons. This is because cotyledons constitute the highest proportion in the seed (18).

Effects of Processing and Storage

Processing significantly affects the retention and distribution of isoflavone isomers in soybeans and soyfoods. Wang and Murphy (19) monitored contents of individual isomers as well as total isoflavones in intermediate products after each step of processing during preparation of soymilk and tofu, tempeh, and soy protein isolate. They found that the processing steps causing significant ($p < .05$) losses of isoflavones are coagulation (44%) in tofu processing, soaking (12%) and heating (49%) in tempeh production, and alkaline extraction (53%) in soy protein isolate preparation. In contrast, fermentation, defatting, and dehulling did not cause significant loss of isoflavones. The observation that isoflavone loss was not significant in okara during tofu making suggests that the compound is mainly associated with soluble proteins rather than insoluble carbohydrates.

Coward *et al.* (7) analyzed isoflavone β -glucoside conjugates and aglucones in various foods and ingredients derived from soybeans. Their results reveal that most Asian soyfoods as well as Western soy ingredients, when not diluted by the addition of nonsoybean components or extracted with aqueous alcohol, have total isoflavone concentrations in the range of 1.33–3.83 mg/g dry weight. These levels are close to those found in the intact soybeans. Fermented soyfoods, which are usually prepared by mixing soy with other components such as barley, rice, and wheat, contained isoflavones at lower concentrations, ranging from 0.36–1.38 mg/g dry weight. Other soy-based products, such as soy sauce and frozen flavored soymilk, had much lower concentrations of isoflavones, with a range of 0.02–0.36 mg/g dry matter. In addition, Asian fermented soyfoods contain predominantly isoflavone aglucones, whereas in nonfermented soyfoods or ingredients of both American and Asian origin isoflavones are present mainly as β -glucoside conjugates. These findings were confirmed by Wang and Murphy (13), who quantified 12 isoflavone isomers in 29 commercial soyfoods, and by a later study by Coward *et al.* (20).

Toasted soybean meal appears to have similar levels of phytoestrogens as the raw seed, indicating that toasting has little effect on isoflavone content (19). Extrusion cooking was found to cause some loss in total isoflavone content (up to 24% reduction) as well as conversion of isomers (21,22).

Many studies indicated transformation of isoflavone isomers during processing. Wang and Murphy (19) found that in the production of tempeh, soymilk, and tofu, malonyldaidzin and malonylgénistin decreased after soaking and cooking. This was accompanied by increases in acetyldaidzin and acetylgenistin. Tempeh fermentation

caused increases in daidzein and genistein, apparently resulting from fungal enzymatic hydrolysis of isoflavone glucosides. In protein isolate processing, alkaline extraction also led to hydrolysis of isoflavone glucosides, resulting in not only loss of total isoflavones but also increases in daidzein and genistein. Furthermore, Barnes *et al.* (23) found that soybeans and defatted soy flour, each of which had been minimally heated during preparation, contained mostly isoflavone 6''-*O*-malonylglucoside conjugates. Soymilk, tofu, and soy molasses, each of which had been heated to 100°C during preparation, contained mostly isoflavone β -glucosides. Toasted soy flour and isolated soy protein had moderate amounts of each of the isoflavone conjugates. Apparently, malonylglucoside conjugates are thermally unstable, and are converted to their corresponding isoflavone glucosides at a high temperature. The de-esterifying reaction was presumably a result of transesterification of the ester linkage between the malonate or acetate carboxyl group and the 6''-hydroxyl group of the glucose moiety, yielding methyl malonate or methyl acetate and the isoflavone glucoside.

The same group (20) later reported similar findings for an expanded list of soyfoods. In addition, they found that alcohol-washed soy concentrate contained few isoflavones. Isolated soy protein and textured vegetable protein consisted of a mixture of all three types of isoflavone conjugates. Baking or frying of textured vegetable proteins at 190°C and baking of soy flour in cookies did not alter total isoflavone content, but there was a steady increase in β -glucoside isoflavones at the expense of the 6''-*O*-malonyl- β -glucoside conjugates, the main form in nonheated soy samples.

It can be concluded that during processing, some steps decrease total content of isoflavones while others (such as heating, defatting, and fermentation) show little or no effect. Yet, conversions of isomers prevail during many steps of processing, even including certain steps, such as heating and fermentation, that have little or no effect on the total isoflavone content.

Storage also causes changes in isoflavones. Eisen *et al.* (24) studied the stability of isoflavones in soymilk stored at elevated and ambient temperatures and found that genistin loss with time showed typical first-order kinetics. At early stages of soymilk storage at 80–90°C, the 6''-*O*-acetyldaidzin concentration increased, followed by a slow decrease.

Effect of Assay Methods

Isoflavones are commonly determined by high-performance liquid chromatography (HPLC) after extraction from test samples with an aqueous organic solvent (10,11,13,23,25–27). A reverse-phase HPLC column and a UV detector are normally required, along with a gradient solvent solution as the mobile phase. However, capillary zone electrophoresis has also been used (14).

There have been variations in extraction conditions among studies. Extractants that have been used include 70% aqueous ethanol (10), 80% aqueous methanol (23), and 80% aqueous acetonitrile containing 0.1% HCl (11,14,23). The extraction time

has ranged from 2 to 24 h and the extraction temperature from refrigeration temperature to 80°C.

Kudou *et al.* (10) reported that when the samples were extracted at 80°C instead of room temperature, malonylated isoflavone glucosides in 70% alcohol extracts from both soybean hypocotyl and cotyledons decreased significantly as glucosides increased. Later, Barnes *et al.* (23) confirmed the finding and found that maximum recovery of the isoflavones from soyfood samples was obtained by tumbling for 2 h at room temperature or 60°C and that there were no significant differences between the use of 80% aqueous methanol and 80% aqueous acetonitrile containing 0.1% HCl. Among the variables related to extraction, temperature has been shown to exert a significant effect on final results with respect to both total isoflavone content and isomer composition. The observed effect of extraction temperature prior to sample analysis on the content and composition of isoflavones was attributed to the heat-induced de-esterifying reaction of malonylglucoside conjugates; Barnes *et al.* (23) recommended that extraction at higher temperatures be avoided. Coward *et al.* (20) also found that hot alcohol extraction de-esterified isoflavone conjugates. Kao and Chen (27) reported that the highest yield of isoflavones was achieved by using defatted soybean powder as raw material, followed by shaking extraction for 2 h with a mixture of acetone and 0.1 M HCl as the solvent.

Furthermore, differences in analytical methods and reporting of isomeric conversions can also contribute significantly to variation in isoflavone values found in the literature. In some studies, total isoflavone is expressed as the sum of all 12 isomers (13). In other studies, only free (aglucone) or bound (conjugated) forms are tested and expressed (7,28). In still other studies isoflavones are hydrolyzed to their aglucone forms or the amount is normalized by molecular weight to the aglucone forms (19). In the later case, because the molecular weight of the glucosides is 1.6 to 1.9 greater than that of the aglucones, the reported total isoflavone amount can be significantly less than the value of non-normalized data (15).

When the amount is adjusted to corresponding aglucones, the concentrations for total daidzein, genistein, and glycitein have a range of 0.20–2.06, 0.32–2.68, and 0.11–1.07 mg/g raw seed, respectively (19,28). When the total isoflavone content is expressed without normalization to aglucones, a range as wide as 0.44–9.10 mg/g raw seed among 319 soybean cultivars tested was reported (16).

Database on the Isoflavone Content of Foods

A few years ago, the Food Composition Laboratory and the Nutrient Data Laboratory of the Agricultural Research Service (ARS), the U.S. Department of Agriculture (USDA), and the Department of Food Science and Human Nutrition of Iowa State University (ISU) started a collaborative effort to develop a database of isoflavones in food. Data for isoflavone contents of foods were collected from scientific articles in peer-reviewed journals. Additional data were generated through sampling soy-containing foods and subsequently analyzing them at ISU. The glucoside forms of

the isoflavones are converted to free forms (aglucones) using appropriate ratios of molecular weights. Values expressed on a dry weight basis were converted to wet weight basis either by using given moisture content or by assuming commonly expected moisture content for that particular food. The table contains mean values, standard errors of the means (SEM), and minimum and maximum values for the individual aglucone forms (daidzein, genistein, and glycitein). Total values were given if values were available for at least daidzein and genistein. The first database was released in 1999. An updated version was released in 2002.

The database is available on the USDA's website (29). Varying contents of isoflavones in different soybean varieties and soy food products shown in the database further confirm the effects of genotypes, growing years, growing locations, and processing and assay methodology. For details, refer to the website (29), Murphy *et al.* (15), and Song *et al.* (26).

Physiological Effects on Humans and Animals

The major soybean isoflavone aglucones, genistein and daidzein, have been identified for many decades (8). Originally, research regarding physiological effects of isoflavones was limited to their estrogen-like activity (30), interference with mineral metabolism, and growth inhibition (31). Furthermore, isoflavones have been shown to be partially responsible for an objectionable aftertaste associated with consumption of soy-based products (10,32,33). This aftertaste is characterized as being sour, bitter, and/or astringent. From this perspective, the presence of isoflavones is undesirable, and they should be eliminated or reduced in soy products (18).

Yet, later researchers have shown many positive effects of isoflavones. It has recently been recognized that the isoflavones contained in vegetable protein materials such as soybeans have medicinal value. Isoflavones have been shown to possess antioxidant and antifungal activity (34), and, more importantly, to act as anticarcinogens (35).

Research has revealed many possible health benefits that may be achieved from the consumption of isoflavones. Under certain experimental conditions isoflavones have been shown to prevent certain types of cancer, reduce bone loss, and alleviate menopausal symptoms. Thus, isoflavones, together with certain other trace compounds present in plants, have been dubbed "phytochemicals." Although they are not classified officially as nutrients, these compounds reportedly affect human health as much as vitamins and minerals do (36). Thus their presence in food is mostly desirable. A very large and growing body of data is available in recent literature on the physiological effects of soy isoflavones. In this section, the health benefits of isoflavones are briefly reviewed. For more details, readers are encouraged to consult recent review papers on the subject, notably Setchell and Cassidy (1), Setchell *et al.* (37), and Messina (5).

Reduction in Coronary Heart Disease Risk

Coronary heart disease (CHD) is a leading cause of death, especially in the United States and other industrialized nations. Elevated total and low-density lipoprotein

(LDL) cholesterol levels are important risk factors for CHD. In humans, soy protein products can lower serum total cholesterol levels and LDL cholesterol levels when consumed at an average intake level of 47 g soy protein per day (38,39).

Preliminary data suggest that isoflavones, like estrogen, may exert cardioprotective effects *via* direct effects on coronary vessels and other physiological processes involved in the etiology of heart disease, although the data are somewhat inconsistent. Soy isoflavones are potent antioxidants capable of reducing the amount of LDL ("bad") cholesterol that undergoes modification in the body and of inducing vascular reactivity (40). Entry of the modified LDL cholesterol into the walls of blood vessels contributes to the formation of plaques. These plaques cause the blood vessels to lose their ability to function normally. Research with animal (2) and human (41) subjects indicates that isoflavones enhance endothelial function, arterial relaxation, and arterial compliance. In addition, Wiseman *et al.* (42) showed that soyfood consumption reduces the extent to which LDL cholesterol is oxidized. For a general review on the coronary effects of isoflavones, readers are encouraged to refer to Nestel (43).

Cancer Prevention

It has also been suggested that isoflavones have the ability to play a role in the prevention of certain cancers. Japanese women who have consumed diets rich in isoflavones appear to have a very low incidence of breast cancer (44). Studies in animals also show that the addition of soy or isoflavones to a standard laboratory diet reduces number of tumors per animal by 25–50% (45–47). In contrast to animal studies, Asian epidemiological studies provided little support for the notion that adult consumption of soy reduces postmenopausal breast cancer risk (48). One hypothesis is that early soy intake is protective against the later development of breast cancer. In support of this hypothesis, Shu *et al.* (49) conducted a study involving approximately 1,500 experimental subjects and 1,500 controls. Women from Shanghai were asked about their soy consumption during the teenage years (age 13–15). It was found that those women who consumed on average approximately 11 g of soy protein per day during the teenage years were 50% less likely to develop breast cancer as compared to women who rarely (<2 g soy protein/day) consumed soy as teenagers. Adult soy intake did not affect these results.

Soy intake may also help to explain why although Japanese men do develop prostate cancer they rarely die from it (44). Preventing small prostate tumors, often referred to as latent cancer, from progressing to the larger tumors that are capable of metastasizing and thus are potentially life-threatening is the key to reducing prostate cancer mortality. Griffiths (50) reported that isoflavones prevented latent prostate cancer from progressing to the more advanced forms of this disease, and Peterson and Barnes (35) showed that genistein inhibits the growth of hormone-dependent and -independent prostate cancer cells *in vitro*. Both genistein and isoflavone glucosides inhibit the growth of both chemically-induced prostate tumors and prostate tumors in rodents implanted with prostate cancer cells (51). In newer studies with

human subjects, 50–70% of the 40 patients with uncontrolled prostate cancer, as determined by rising prostate specific antigen (PSA) levels, favorably responded (as judged by PSA levels) to a daily supplement of 120 mg of isoflavones (3,52). Based on these and other findings, the American Cancer Society includes eating soyfoods as one of seven steps men can take to reduce their risk of developing prostate cancer.

Women's Health

It is thought that at least some of the soy isoflavone fractions are especially beneficial for women in general since soy is a source of plant or vegetable estrogen. It is thought that plant or vegetable estrogen provides many of the advantages and avoids some of the alleged disadvantages of animal estrogen. In fact, the estrogen-like effects of isoflavones in combination with the low reported frequency of hot flashes in Japan prompted investigation of the effect of soy on menopausal symptoms. In a recent review, 19 trials involving over 1,700 women were identified. Six trials were excluded from the analysis for methodological reasons. Based on a simple regression analysis of the remaining data, there was a statistically significant ($P = 0.01$) relationship between initial hot flash frequency and treatment efficacy (53).

Bone Health

Soy isoflavones are actively studied for their effects on maintaining and improving bone health. Women can lose up to 15% of their total bone mass in the early years following the onset of menopause. This loss can be quite detrimental, particularly to women who enter menopause with weaker bones. Emerging research shows that isoflavones appear to play a role in both preventing bone loss and increasing bone density (54,55). In addition, several other studies have examined the effect of soy or isoflavones on markers of bone resorption and/or formation (56,57). Overall, the results of clinical studies are encouraging. Speculation about the skeletal benefits of isoflavones was based initially on the similarity in chemical structure between isoflavones and estrogen. This is supported by a recent study that found genistein to be as effective as conventional hormone replacement therapy in preventing bone loss at the spine and hip in postmenopausal women (58). Recent reviews are available on the subject (59,60).

Extraction, Isolation, Purification, and Commercial Production

While most soyfoods contain some quantity of isoflavones, traditionally, individuals have been limited in their use of soyfoods to increase their levels of dietary isoflavones because (a) the number and variety of soyfoods is limited, especially in the U.S. marketplace; (b) the natural level may not be sufficient to meet the serving range needed to have a physiological impact; and (c) natural flavors and color of some soy products have been described by some people as being bitter and unappe-

tizing. Furthermore, some by-products of soy processing, such as soy molasses, contain relatively high concentrations of isoflavones. Recovery of isoflavones would improve end-use value of these products. Therefore, it is desirable to extract and concentrate the isoflavone fraction from the source material. This process is preferable for making isoflavones into pills, tablets, capsules, liquids, and food ingredients that may be ingested without having to taste the original food product. It is desirable to use the isoflavones as supplements in foods, beverages, medical foods, health bars, and certain other dietary supplement products. As a result, many companies have introduced concentrated forms of isoflavones that can be used as an ingredient in foods or beverages or incorporated into dietary supplements (6).

The remaining sections of this chapter provide an overview of the techniques that have been evolved over recent years for extracting, isolating, concentrating, and purifying isoflavones from plant materials, particularly soy material. Techniques for converting certain isoflavone isomers to more potent forms, such as from glucoside or conjugate forms to aglucones, are also discussed. There are countless publications covering these subjects; not surprisingly, because of excellent commercial value and profitability of isoflavone products, most of the publications come from patent literature.

Starting Material

Soy molasses, defatted soy flakes or flour, and soy germs are commonly used as starting material for isolating and concentrating isoflavones. Other plant materials that are rich in isoflavones, such as red clover, alfalfa, flax, cocoa, tea, and kudzu root, are also used as starting materials.

Soy molasses is by far the most common starting material. In a conventional process for the production of a soy protein concentrate in which soy flakes are extracted with an aqueous acid or an aqueous alcohol to remove water-soluble materials from the soy flakes, a large portion of the isoflavones is solubilized in the extract. The extract of water-soluble materials, including the isoflavones, is soy molasses. The soy molasses is a by-product material in the production of soy protein concentrate that is typically discarded. Soy molasses, therefore, is an inexpensive and desirable source of isoflavones, provided that the isoflavones can be separated from the soy molasses (see Chapter 9).

Soy germs are also known as hypocotyls. As mentioned earlier, the concentration of the total isoflavones in soy germs is 5.5–6 times higher than that in cotyledons (10,18). During soybean processing, germs are broken away at the cracking and dehulling stage, and can be collected for isoflavone production or used directly as an ingredient for dietary supplements. If not collected, soy germs go with hulls and end up in animal feed. A U.S. patent was issued to Kelly (61) for the use of soy germs as dietary supplements.

Other legumes such as soybean flour may be used for enrichment of phytoestrogens, but the substantially poorer (~10%) yield of isoflavones compared to clovers means that the manufacturing costs are substantially greater and there are substantially greater amounts of waste products, which require disposal or further treatment

for reuse as a foodstuff. An alternative, however, to the use of whole soy for this purpose is to use the hull and hypocotyl (or germ) of the whole soybean. The hull and hypocotyl represent only a small proportion by weight (8% and 2%, respectively) of the intact bean. However, the coumestrol content of soy is concentrated in the hull, and the daidzein content of soy is concentrated in the hypocotyl. The two cotyledons that compose the bulk of the soybean (90% by weight) contain the bulk of the genistein content of soy. During standard processing of soybeans, the hulls, being a fibrous component with little or no perceived nutritional value, normally are separated and removed by physical means. The hypocotyls become separated following the splitting of the cotyledons, and while these currently generally are not deliberately isolated, they may be separated and isolated by passing the disturbed soybeans over a sieve of sufficient pore size to selectively remove the small hypocotyl. The hypocotyl contains approximately 1.0–1.5% isoflavones by weight (95% daidzein, 5% genistein). The raw hypocotyl and hull material can be ground or milled to produce, for example, a dry powder or flour that then could be either blended or used separately as a dietary supplement in a variety of ways including, for example, as a powder, in a liquid form, in a granulated form, in a tablet, or in an encapsulated form, or added to other prepared foodstuffs. Alternatively, it could be further processed to yield an enriched extract of phytoestrogens. Either or both of these materials also could be added to other leguminous material such as clover to provide the desired product.

General Extraction and Purification

In one of the earlier publications regarding soy isoflavones, Walter (8) reported a method for the extraction and isolation of genistin and its aglucone, genistein, from soybeans. Briefly, defatted soybean flakes, which had been extracted with hexane, were twice extracted using methanol. Acetone was added to the combined methanolic extracts to precipitate some of the phosphatides and other impurities. The supernatant was decanted and two volumes of water were added to precipitate out the genistin. Multiple recrystallizations were then performed to purify the genistin. Ohta *et al.* (62) disclosed a method of isolating and purifying isoflavones from defatted soybeans whereby the defatted soybeans are extracted with ethanol and the resulting ethanol extracts are treated with acetone and ethyl acetate. The ethyl acetate extract is then fractionated over silica gel and Sephadex LH-20 columns followed by multiple recrystallizations. Farmakalidis *et al.* (63) reported that acetone mixed with 0.1 N HCl was superior to 80% methanol as an extraction solvent. The subsequent isolation procedure followed that of Ohta *et al.* (62).

Fleury and Magnolato (64) described a method for preparing an impure extract of two specific isoflavones, daidzin malonate and genistin malonate. The method involves, among other steps, mixing defatted soy material with 80% aqueous methanol, filtering, and drying; adjusting pH multiple times with, among other chemicals, hydrochloric acid and sodium hydroxide, and extracting with an organic solvent, such as butanol. Chaihorsky (65) described a process based on chromatography using strong cation-exchange resins. Dobbins and Konwinski (66) reported a

process for making an isoflavone concentrate product from soybeans that includes diluting soy molasses to about 10–30% solids, separating undissolved solids from the diluted soy solubles, such that the separated solids have at least 4% isoflavones by weight of dry matter. The concentrate can then be further concentrated to at least 40% isoflavones by weight of dry matter by adjusting pH and temperature and extracting with solvents. The soy isoflavone concentrate products are then used in liquid or dry beverages, food, or nutritional products.

Zheng *et al.* (67) reported an improved method for extracting, isolating, and purifying isoflavones from a plant material. It is a three-step process. First a biomass containing isoflavones is mixed with a solvent. Second, the extract is fractionated using a reverse-phase matrix in combination with a step-gradient elution. The resulting fractions eluted from the column contain specific isoflavones, which are later crystallized. The purified isoflavone glucosides may then be hydrolyzed to their respective aglucones.

Enrichment and Conversion

Shen (68) described a method for making an aglucone-enriched vegetable protein fiber. The steps include solubilizing isoflavones from soy flour by forming a slurry with an extractant, such as sodium, potassium, or calcium hydroxide, adjusting the pH to the proteins' isoelectric point of 6.7–9.7, reacting the slurry with the enzyme β -glucosidase to convert the glucone isoflavones in the slurry to aglucone isoflavones, and recovering the fiber fraction from the slurry by centrifugation or similar means to provide an aglucone-enriched fiber.

In a series of patents, Waggle *et al.* (69) disclosed a process to recover an isoflavone-enriched material from soy molasses, convert isoflavone conjugates in soy molasses to isoflavone glucosides and aglucone isoflavones, and then recover an isoflavone glucoside-enriched material and an aglucone isoflavone-enriched material from soy molasses. The method consists of providing a soy molasses material containing isoflavones, and separating a cake from the soy molasses material at a pH and a temperature sufficient to cause a majority of the isoflavones to be contained in the cake. Preferably the pH is about 3.0–6.5 and the temperature is about 0–35°C. during the separation. The cake is an isoflavone-enriched material. The material can be further processed to produce isoflavone glucoside-enriched material or isoflavone aglucone-enriched material. In this case, an aqueous slurry is formed of the isoflavone-enriched material. The slurry is treated at a temperature of about 2–120°C and a pH of about 6–13.5 for a time sufficient to convert isoflavone conjugates in the isoflavone-enriched material to isoflavone glucosides. A cake of isoflavone glucoside-enriched material may then be separated from the slurry. Alternatively, an enzyme capable of cleaving 1,4-glucosidic bonds is added to the isoflavone glucosides in the slurry at a temperature of about 5–75°C and a pH of about 3–9 for a time sufficient to convert the isoflavone glucosides to aglucone isoflavones. A cake of aglucone isoflavone-enriched material may be separated from the slurry.

Kelly *et al.* (70) reported an improved method in which isoflavone-containing plant material (such as defatted soy material or soy germ), water, an enzyme that cleaves isoflavone glucosides to the aglucone form, and an organic solvent are mixed to allow isoflavones to partition into the organic solvent component, and thereafter isoflavones are recovered from the organic solvent component. The enzyme used to cleave isoflavone glucosides to the aglucone form is a β -glucanase or a combination of β -glucanase and β -xylanase.

Although various techniques have been proposed to isolate, convert, and concentrate isoflavones from plant materials, essentially there are two distinct methods. The first method involves the conversion of the water-soluble aglucone form to the water-insoluble aglucone form to facilitate the subsequent extraction of the aglucones in a suitable organic solvent. This conversion step is described as being achieved in one of two ways: either (a) through hydrolysis by exposure to vigorous heating (typically 80–100°C) at low pH (25), or (b) by exposure to an enzyme (glucose hydrolase, β -glucosidase, or β -glucuronidase) that specifically cleaves the β -glycosidic linkage with the sugar moiety. The enzyme can be added to the reaction or the naturally occurring β -glucosidase within the plant can be activated through mild heating. After hydrolysis, the aqueous phase is separated from undissolved plant material to facilitate the next step. Once the conversion of the glucone to the aglucone form is achieved, the aqueous mixture is mixed with an organic (and water-immiscible) solvent. The aglucones are extracted into the organic solvent phase and subsequently recovered, due to their insolubility in water.

The second method involves initial water extraction of isoflavones in their natural form; they either are retained in this form or subsequently converted to their aglucone form. The techniques described for this approach involve adding the ground plant material to water. Over a period of time (several hours to several days) the naturally-occurring glucosidic forms of the isoflavones dissolve in the aqueous phase. After separating the undissolved plant material from the aqueous phase, the isoflavones in the aqueous phase can be converted to the aglucone form by any of the methods mentioned previously and subsequently recovered.

Separation and Recovery of Both Isoflavones and Protein Materials

Since isoflavones have been associated with the bitter, beany taste of legumes that contain significant amounts of the compounds, it is desirable to separate and recover both an isoflavone-depleted, pleasant-tasting protein material and the health-beneficial isoflavones from a plant material containing both isoflavones and protein.

Many reported methods, while satisfactory for separating and purifying isoflavones from a plant material, do not provide a method for recovering both a purified protein material and isoflavones from a plant material containing isoflavones and protein. Furthermore, many methods utilize an alcohol solvent to extract isoflavones from the plant material. Plant proteins such as soy protein are substantially insoluble in alcohol solutions, and will be left as a by-product residue from the alcohol extraction, along with other plant materials insoluble in alcohol, such as plant fiber materials.

Iwamura (71) provided a process for separating plant proteins and flavonoids, including isoflavones, from a plant material containing both. A plant material is extracted with an aqueous alkaline solution to form an extract containing the flavonoids and protein, and the extract is separated from unextractable and insoluble plant materials. The extract is applied on a nonpolar or slightly polar adsorbent resin as it is, or after being acidified, to adsorb the flavonoids on the resin. Acidification causes the protein to be precipitated from the extract. If acidified, the precipitated protein is separated from the extract prior to application of the extract on the resin. After applying the extract on the resin, the resin is eluted with water and the eluant is collected to provide an aqueous solution containing carbohydrates. The water eluant is acidified to precipitate and separate the protein if the protein was not precipitated and separated from the extract prior to application on the resin. The flavonoids are then separated from the resin by elution with a polar solvent such as methanol or ethanol and collection and concentration of the eluant. Using this method, isoflavones and carbohydrates/protein are not separated cleanly, due to the nature of the isoflavones and the resin and eluants used in the process.

Bates and Bryan (72) disclosed an improved method of separating and collecting isoflavones and protein from a plant material that can be efficiently and economically performed on a commercial scale. The method involves separating and collecting isoflavones and a plant protein by placing a clarified plant protein extract containing isoflavones and protein in contact with a polar ion-exchange resin; allowing the isoflavones to bind with the polar ion-exchange resin; separating and recovering an isoflavone-depleted protein extract from the ion-exchange resin; and then separating and recovering the isoflavones from the ion-exchange resin. In a preferred embodiment of this process, the separated and recovered isoflavones are converted to their aglucone forms.

High Concentrations

In a series of patents, Gugger and Grabiel (73) reported a method that was claimed to be able to produce highly enriched isoflavone products containing either a wide range of soy isoflavones or highly purified genistin gained from an ethanol extract of defatted soybean flakes. The temperature-sensitive differential of solubility of various isoflavone fractions is used to initially separate the fractions, preferably by heating an aqueous soy molasses or soy whey feed stream. The temperature of the feed stream is selected according to the temperature at which a desired isoflavone fraction or fractions become soluble. Then, the heated feed stream is passed through an ultrafiltration membrane in order to concentrate the isoflavones. The feed stream is put through a resin adsorption process. The isoflavone fractions are treated with either reverse osmosis or ultrafiltration (or both) to complete solvent removal and to achieve a higher isoflavone concentration in the end product. Then, the feed stream is dried, preferably by spray drying, to produce dry particles. The resulting product was claimed to be a combination of isoflavone fractions, which have a neutral color and a bland flavor, and which together provide a profile especially directed to specific health problems. The process can produce isoflavone materials of greater concentration, so that smaller

quantities of a supplement deliver the same amount or more of the desired isoflavones. It can provide a supplement that may be included in a great variety of foods and beverages. The product is typically about 30–50% isoflavones on a dry solids basis.

Empie and Gugger (74) patented a method for preparing and using isoflavones from soy and other plants (and the resulting composition) for a dietary supplement for treatment of various cancers, pre- and postmenstrual syndromes, and various other disorders. The composition is obtained by fractionating a plant source high in isoflavones, lignans, and other phytochemicals such as defatted soybean flakes, soy molasses, soy whey, red clover, alfalfa, flax, cocoa, tea, or kudzu root. These may be fractionated along with or in combination with other plants known to be high in the various isoflavones, lignans, saponins, catechins, and phenolic acids. The fractionation results in substantially removing water, carbohydrates, proteins, and lipids from the source material. Other extraction processes, which may be used alone or in combination, include differential solubility, distillation, solvent extraction, adsorptive means, differential molecular filtration, and precipitation. The composition is in a concentrated form to be delivered in an easy-to-consume dosage, such as a pill, tablet, liquid, or capsule, or in a food supplement such as a health bar.

Hilaly *et al.* (75), in a U.S. patent application, disclosed an invention that provides a simple and effective method for producing high-purity isoflavones from soy solubles. The process comprises two steps: (a) subjecting the plant material to a primary chromatographic step to obtain an isoflavone-enriched fraction and (b) subjecting the isoflavone-enriched fraction to a second chromatographic step. More specifically, the process comprises the following steps: (a) heating an aqueous plant starting material to a constant temperature selected on the basis of the aqueous solubility of at least one desired isoflavone fraction that is to be recovered; (b) passing the heated starting material through an ultrafiltration membrane to obtain a plant material permeate, the membrane having a cut-off that passes at least one desired isoflavone fraction; (c) treating the permeate with an adsorptive material; (d) washing the adsorptive material in water; (e) eluting at least one adsorbed isoflavone fraction from the water-washed adsorptive material with aqueous alcohol to obtain an isoflavone-enriched fraction; (f) adsorbing the isoflavone-enriched fraction in a secondary chromatography with an adsorptive material; (g) eluting, with one or more series of at least one bed volume of aqueous alcohol, at least one isoflavone fraction from the secondary chromatography; and (h) evaporating the aqueous alcohol used during the elution in order to promote the crystallization of at least one isoflavone fraction.

Principles and Limitations of Current Methods

As just discussed, countless methods and techniques are available to extract, isolate, and purify isoflavones. Yet, they can be categorized based on several general principles (or approaches). One group is based on extraction and then precipitation. Another group is based on precipitation and then extraction or separation. The third group is based on the use of chromatography or other means either before or after solvent extraction to separate or concentrate isoflavones.

Although some of the methods previously discussed are used for commercial production of isoflavones, almost all the reported methods are affected by one or more of the following disadvantages, which greatly reduce the commercial viability of these processes. First, most reported processes include multiple steps; some require multiple chromatography columns and many are too cumbersome for the production of isoflavones on an industrial scale. Second, many use vigorous treatments such as heating, strong acid, strong alkali, and/or various organic solvents, which can have negative environmental impact and decrease end-use values of remaining components in the original material. Third, some use high-cost hydrolyzing enzymes. The problem with multiple steps and various solvents and other factors in reported processes is that the disclosed laboratory-scale processes are not easily scaled up to an efficient commercial process, where considerations such as disposal of various solvents play an important role in the overall feasibility of the process. Furthermore, for methods using multiple chromatography columns, the eluants utilized by various researchers typically separate the isoflavones from other compounds present in the plant extract. However, further separation techniques involving chromatography are required to separate the individual isoflavone compounds. These separation techniques necessitate the continuous monitoring of the eluant as it runs off the column, thus making it possible to collect those fractions of eluant that contain a particular isoflavone. Other disadvantages of many laboratory processes are low yields of isoflavones and the inability to make a high-purity product. A typical purity level associated with many of these methods is only in the 4–50% range. Because of disadvantages associated with many reported procedures, high capital costs and high running costs are associated with large-scale extraction of isoflavones in commercial quantities. There is still a need, therefore, for a process and procedure for isolating and purifying isoflavones from isoflavone-containing biomass in a commercially viable manner.

Safety and Emerging Findings about Soy Isoflavones

Soy isoflavones have been a component of the diet of certain populations for centuries. The consumption of soy generally has been considered beneficial, with a potential protective effect against a number of chronic diseases. Yet, because of their estrogenic activity, there has been concern about the safety of consuming isoflavones, particularly for infants and in the case of overconsumption by general populations. Several negative effects have been postulated. A lead review article (76) examined the literature associated with the safety of soy isoflavones. The conclusion was that whereas results in some studies are limited or conflicting, when reviewed in its entirety the current literature supports the safety of isoflavones as typically consumed in diets based on soy or soy-containing products.

Yet, in what could be seen as a blow to the fast-growing market for soy nutraceutical products, several new studies (77–79) suggest that processing soy materials into concentrated isoflavone form for use in supplements and food products could seriously reduce its cancer-fighting ability. In one study (79), mice were fed soy flour or mixed

isoflavone diets, each containing equal concentrations of the soy isoflavone genistein. This allowed the researchers to determine the influences that various bioactive soy compounds had on genistein's ability to stimulate estrogen-dependent breast tumor growth. Results show that as bioactive compounds were removed, there was an increase in estrogen-dependent tumor growth. Bennink *et al.* (77) showed inhibition of colon cancer by soy flour but not by genistin or a mixture of isoflavones, while Keinan-Boker *et al.* (78) concluded that plant estrogens, such as isoflavones or lignans, do not appear to have any effect on reducing breast cancer risk in Western women when ingested as dietary supplements.

Soy consumption has been correlated with low rates of breast cancer in Asian populations, but soyfoods in Asia are made from minimally processed soybeans or from defatted, toasted soy flour, which is quite different from soy products consumed in the West. Isoflavone-containing products consumed in the United States may have lost many of the biologically active components in soy, and these partially purified isoflavone-containing products may not have the same health benefits as whole-soy foods. In other words, the healthy properties of the soy used widely in Asian cuisine—on which the burgeoning popularity of the soy-based health food industry is founded—may be largely destroyed by the processing techniques used in the West.

Furthermore, new studies show that genistein, when present either in purified form (80) or in isolate soy protein (81) can stimulate growth of estrogen-dependent tumors in athymic mice in a dose-dependent manner. This has created some controversy on the role of soy isoflavones, particularly genistein, in breast cancer prevention.

It is evident that research on the health effects of phytoestrogens, including isoflavones, is rather complicated, and in many cases, results are either inconclusive or inconsistent among different studies. Therefore, more research is definitely needed.

References

1. Setchell, K.D.R., and A. Cassidy, Dietary Isoflavones: Biological Effects and Relevance to Human Health, *J. Nutr.* 129:758S–767S (1999).
2. Anthony, M.S., Soy and Cardiovascular Disease: Cholesterol Lowering and Beyond, *J. Nutr.* 130:662S–3S (2000).
3. Hussain, M., F.H. Sarkar, Z. Djuric, *et al.*, Soy Isoflavones in the Treatment of Prostate Cancer, *J. Nutr.* 132:575S–576S (2002).
4. Messina, M., Soyfoods and Their Role in Disease Prevention and Treatment, in *Soybeans: Chemistry, Technology, and Utilization*, Kluwer Academic Publishers, New York, 1997, pp. 442–477.
5. Messina, M., Potential Public Health Implications of the Hypocholesterolemic Effects of Soy Protein, *Nutr.* 19:280–281 (2003).
6. Jarvis, L., Soy Isoflavones Set to Blossom as Consumer Interest Grows, *Chemical Market Reporter*, September 9, 2002, pp. 12, 14.
7. Coward, L., N.C. Barnes, K.D.R. Setchell, and S. Barnes, Genistein, Daidzein, and Their beta-Glycoside Conjugates: Antitumor Isoflavones in Soybean Foods from American and Asian Diets, *J. Agric. Food Chem.* 41:1961–1967 (1993).
8. Walter, E.D., Genistin (an Isoflavone Glycoside) and Its Aglucone, Genistein from Soybeans, *J. Am. Chem. Soc.* 63:3273–3276 (1941).

9. Eldridge, A., and W. Kwolek, Soybean Isoflavones: Effect of Environment and Variety on Composition, *J. Agric. Food Chem.* 31:394–396 (1983).
10. Kudou, S., Y. Fleury, D. Welti, D. Magnolato, T. Uchida, K. Kitamura, and K. Okubo, Malonyl Isoflavone Glycosides in Soybean Seeds (*Glycine max* Merrill), *Agric. Biol. Chem.* 55:2227–2233 (1991).
11. Wang, H.-J., and P.A. Murphy, Isoflavone Composition of American and Japanese Soybeans in Iowa: Effects of Variety, Crop Year and Location, *J. Agric. Food Chem.* 42:1974–1677 (1994).
12. Hoeck, J.A., W.R. Fehr, P.A. Murphy, and G.A. Welke, Influence of Genotype and Environment on Isoflavone Contents of Soybean, *Crop Sci.* 40:48–51 (2000).
13. Wang, H.-J., and P.A. Murphy, Isoflavone Content in Commercial Soybean Foods, *J. Agric. Food Chem.* 42:1666–1673 (1994).
14. Aussenac, T., S. Lacombe, and J. Dayde, Quantification of Isoflavones by Capillary Zone Electrophoresis in Soybean Seeds: Effects of Variety and Environment, *Am. J. Clin. Nutr.* 68:1480S–1485S (1998).
15. Murphy, P.A., K. Barua, and T.T. Song, Soy Isoflavones in Foods: Database Development, in *Functional Foods for Disease Prevention I*, ACS Symposium Series, 701, edited by T. Shibamoto, J. Terao, and T. Osawa, American Chemical Society, Washington, D.C., 1998, pp. 138–149.
16. Kikuchi, A., *et al.*, Genetic Diversity and Inheritance of Isoflavone Contents in Soybean Seeds, in *Proceedings, the Third International Soybean Processing and Utilization Conference*, October 15–20, 2000, Tsukuba, Japan, Korin Publishing Co., Tokyo, Japan, pp. 59–60.
17. Carrao-Panizzi, M.C., K. Kitamura, A.D. Beleia, and M.C.N. Oliveira, Influence of Growth Locations on Isoflavone Contents in Brazilian Soybean Cultivars, *Breeding Sci.* 48:409–413 (1998).
18. Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, and K. Kitamura, Factors Affecting Isoflavone Content in Soybean Seeds: Changes in Isoflavones, Saponins, and Composition of Fatty Acids at Different Temperatures during Seed Development, *J. Agric. Food Chem.* 43:1184–1192 (1995).
19. Wang, H.-J., and P.A. Murphy, Mass Balance Study of Isoflavones during Soybean Processing, *J. Agric. Food Chem.* 44:2377–2383 (1996).
20. Coward, L., M. Smith, M. Kirk, and S. Barnes, Chemical Modification of Isoflavones in Soyfoods during Cooking and Processing, *Am. J. Clin. Nutr.* 68:1496S–1491S (1998).
21. Singletary, K., J. Faller, J.Y. Li, and S. Mahungu, Effect of Extrusion on Isoflavone Content and Antiproliferative Bioactivity of Soy/Corn Mixtures, *J. Agric. Food Chem.* 48:3566–3571 (2000).
22. Rinaldi, V.E.A., P.K.W. Ng, and M.R. Bennis, Effects of Extrusion on Dietary Fiber and Isoflavone Contents of Wheat Extrudates Enriched with Wet Okara, *Cereal Chem.* 77:237–240 (2000).
23. Barnes, S., M. Kirk, and L. Coward, Isoflavones and Their Conjugates in Soy Foods: Extraction Conditions and Analysis by HPLC-Mass Spectrometry, *J. Agric. Food Chem.* 42:2466–2474 (1994).
24. Eisen, B., Y. Ungar, and E. Shimoni, Stability of Isoflavones in Soy Milk Stored at Elevated and Ambient Temperatures, *J. Agric. Food Chem.* 51:2212–2215 (2003).

25. Wang, K., S.S. Kuan, O.J. Francis, K.M. Ware, and A.S. Carman, A Simplified HPLC Method for the Determination of Phytoestrogens in Soybean and Its Processed Products, *J. Agric. Food Chem.* 38:185–190 (1990).
26. Song, T.T., K. Barua, G. Buseman, and P.A. Murphy, Soy Isoflavone Analysis: Quality Control and a New Internal Standard, *Am. J. Clin. Nutr.* 68:1474S–1479S (1998).
27. Kao, T.H., and B.H. Chen, An Improved Method for Determination of Isoflavones in Soybean Powder by Liquid Chromatography, *Chromatographia* 56:423–430 (2002).
28. Taylor, N.B., R.L. Fuchs, J. MacDonald, A.R. Shariff, and S.R. Padgett, Compositional Analysis of Glyphosate-Tolerant Soybeans Treated with Glyphosate, *J. Agric. Food Chem.* 47:4469–4473 (1999).
29. United States Department of Agriculture, Iowa State University, and the Agricultural Research Service, Database on the Isoflavone Content of Foods, available at www.nal.usda.gov/fnic/foodcomp/data/isoflav/isoflav.html (accessed June 24, 2004).
30. Wong, E., and D.S. Flux, Estrogenic Activity of Red Clover Isoflavones and Some of Their Degradation Products, *J. Endocrinol.* 24:341–348 (1962).
31. Magee, A.C., Biological Responses of Young Rats Fed Diets Containing Genistin and Genistein, *J. Nutr.* 80:151–156 (1963).
32. Huang, A.S., O.A.L. Hsieh, and S.S. Chang, Characterization of the Nonvolatile Minor Constituents Responsible for the Objectionable Taste of Defatted Soybean Flour, *J. Food Sci.* 47:19 (1981).
33. Okubo, K., M. Iijima, Y. Kobayashi, M. Yoshikoshi, T. Uchida, and S. Kudou, Components Responsible for the Undesirable Taste of Soybean Seeds, *Biosci. Biotechnol. Biochem.* 56:99–103 (1992).
34. Fleury, Y., D.H. Welti, G. Phillipossian, and D. Magnolato, Soybean (Malonyl) Isoflavones: Characterization and Antioxidant Properties, in *Phenolic Compounds in Food and Their Effects on Health*, edited by M.-T. Huang, C.-T. Ho, and C.Y. Lee, American Chemical Society, Washington, D.C., 1992, Vol. II, pp. 98–113.
35. Peterson, T.G., and S. Barnes, Genistein and Biochanin A Inhibit the Growth of Human Prostate Cancer Cells, but Not Epidermal Growth Factor Receptor Tyrosine Autophosphorylation, *Prostate* 22:335–345 (1993).
36. Messina, M., V. Messina, and K.D.R. Setchell, *The Simple Soybean and Your Health*, Avery Publishing Group, Garden City Park, New York, 1994.
37. Setchell, K.D.R., M. Nadine, N.M. Brown, P. Desai, L. Zimmer-Nechemias, B.E. Wolfe, W.T. Brashear, A.S. Kirschner, A. Cassidy, and J.E. Heubi, Bioavailability of Pure Isoflavones in Healthy Humans and Analysis of Commercial Soy Isoflavone, *J. Nutr.* 131:1362S–1375S (2001).
38. Anderson, J.W., B.M. Johnstone, and M.L. Cook-Newell, Meta-analysis of the Effects of Soy Protein Intake on Serum Lipids, *N. Engl. J. Med.* 333:276 (1995).
39. Weggemans, R.M., and E.A. Trautwein, Relation between Soy-Associated Isoflavones and LDL and HDL Cholesterol Concentrations in Humans: A Meta-analysis, *Eur. J. Clin. Nutr.* 57:940–946 (2003).
40. Squadrito, F., D. Altavilla, N. Morabito, A. Crisafulli, R. D’Anna, F. Corrado, P. Ruggeri, G.M. Campo, G. Calapai, A.P. Caputi, and G. Squadrito, The Effect of the Phytoestrogen Genistein on Plasma Nitric Oxide Concentrations, Endothelin-1 Levels and Endothelium Dependent Vasodilation in Postmenopausal Women, *Atherosclerosis* 163:339–347 (2002).
41. Nestel, P.J., T. Yamashita, T. Sasahara, S. Pomeroy, A. Dart, P. Komesaroff, A. Owen, and M. Abbey, Soy Isoflavones Improve Systemic Arterial Compliance but Not Plasma Lipids in

Menopausal and Perimenopausal Women, *Arterioscler. Thromb. Vasc. Biol.* 17:3392–3398 (1997).

42. Wiseman, H., J.D. O'Reilly, H. Adlercreutz, *et al.*, Isoflavone Phytoestrogens Consumed in Soy Decrease F(2)-Isoprostane Concentrations and Increase Resistance of Low-Density Lipoprotein to Oxidation in Humans, *Am. J. Clin. Nutr.* 72:395–400 (2000).
43. Nestel, P., Isoflavones: Their Effects on Cardiovascular Risk and Functions, *Curr. Opin. Lipidol.* 14:3–8 (2003).
44. Pisani, P., D.M. Parkin, F. Bray, and J. Ferlay, Estimates of the Worldwide Mortality from 25 Cancers in 1990, *Int. J. Cancer* 83:18–29 (1999).
45. Barnes, S., C. Grubbs, K.D. Setchell, and J. Carlson, Soybeans Inhibit Mammary Tumors in Models of Breast Cancer, *Prog. Clin. Biol. Res.* 347:239–253 (1990).
46. Lamartiniere, C.A., Y.X. Zhao, and W.A. Fritz, Genistein: Mammary Cancer Chemoprevention, in Vivo Mechanisms of Action, Potential for Toxicity and Bioavailability in Rats, *J. Women's Cancer* 2:11–19 (2000).
47. Hewitt, A.L., and K.W. Singletary, Soy Extract Inhibits Mammary Adenocarcinoma Growth in a Syngenic Mouse Model, *Cancer Lett.* 192:133–143 (2003).
48. Trock, B., W. Butler, R. Clarke, and L. Hilakivi-Clarke, Meta-analysis of Soy Intake and Breast Cancer Risk [abstract], *J. Nutr.* 130:690S–691S (2000).
49. Shu, X.O., F. Jin, Q. Dai, *et al.*, Soyfood Intake during Adolescence and Subsequent Risk of Breast Cancer among Chinese Women, *Cancer Epidemiol. Biomarkers Prev.* 10:483–488 (2001).
50. Griffiths, K., Estrogens and Prostatic Disease, *Prostate* 45:87–100 (2000).
51. Pollard, M., and W. Walter, Prevention of Spontaneous Prostate-related Cancer in Lobund-wistar Rats by a Soy Protein Isolate/Isoflavone Diet, *Prostate* 45:101–105 (2000).
52. Hussain, M., M. Banerjee, F.H. Sarkar, Z. Djuric, M.N. Pollak, D. Doerge, J. Fontana, S. Chinni, J. Davis, J. Forman, D.P. Wood, and O. Kucuk, Soy Isoflavones in the Treatment of Prostate Cancer, *Nutr. Cancer* 47:111–117 (2003).
53. Messina, M., and C.L. Hughes, The Efficacy of Soyfoods and Soybean Isoflavone Supplements for Alleviating Menopausal Symptoms Is Positively Related to Initial Hot Flash Frequency, *J. Medicinal Foods* 6:1–11 (2003).
54. Alekel, D.L., A.S. Germain, C.T. Peterson, K.B. Hanson, J.W. Stewart, and T. Toda, Isoflavone-rich Soy Protein Isolate Attenuates Bone Loss in the Lumbar Spine of Perimenopausal Women, *Am. J. Clin. Nutr.* 72:844–852 (2000).
55. Gallagher, J.C., K. Rafferty, V. Haynatzka, and M. Wilson, Effect of Soy Protein on Bone Metabolism, *J. Nutr.* 130:867S (2000).
56. Murkies, A.L., C. Lombard, B.J. Strauss, G. Wilcox, H.G. Burger, and M.S. Morton, Dietary Flour Supplementation Decreases Post-menopausal Hot Flushes: Effect of Soy and Wheat, *Maturitas* 21:189–195 (1995).
57. Wangen, K.E., A.M. Duncan, B.E. Merz-Demlow, *et al.*, Effects of Soy Isoflavones on Markers of Bone Turnover in Premenopausal and Postmenopausal Women, *J. Clin. Endocrinol. Metab.* 85:3043–3048 (2000).
58. Morabito, N., A. Crisafulli, C. Vergara, *et al.*, Effects of Genistein and Hormone-Replacement Therapy on Bone Loss in Early Postmenopausal Women: A Randomized Double-Blind Placebo-Controlled Study, *J. Bone Miner. Res.* 7:1904–1912 (2002).
59. Branca, F., Dietary Phyto-oestrogens and Bone Health, *Proc. Nutr. Soc.* 62:877–887 (2004).
60. Cotter, A., and K.D. Cashman, Genistein Appears to Prevent Early Postmenopausal Bone Loss as Effectively as Hormone Replacement Therapy, *Nutr. Rev.* 61:346–351 (2003).

61. Kelly, G.E., Dietary Supplements Comprising Soy Hypocotyls Containing at Least One Isoflavone, U.S. Patent 6,497,906, December 24, 2002.
62. Ohta, *et al.*, Isoflavonoid Constituents of Soybeans and Isolation of a New Acetyl Daidzin, *Agric. Biol. Chem.* 43:1415–1419 (1979).
63. Farmakalidis, E., and P.A. Murphy, Isolation of 6''-O-Acetylgenistin and 6''-O-Acetyldaidzin from Toasted Defatted Soy Flakes, *J. Agric. Food Chem.* 33:385–389 (1985).
64. Fleury, Y., and D. Magnolato, Process for Obtaining Genistin Malonate and Daidzin Malonate, U.S. Patent 5,141,746, August 25, 1992.
65. Chaihorsky, A., Process for Obtaining an Isoflavone Concentrate from a Soy Extract, U.S. Patent 6,670,632, September 23, 1997.
66. Dobbins, T.A., and A.H. Konwinski, Soy Isoflavone Concentrate Process and Product, U.S. Patent 6,369,200, April 9, 2002.
67. Zheng, B.L., J.A. Yegge, D.T. Bailey, and J.L. Sullivan, Process for the Isolation and Purification of Isoflavones, U.S. Patent 5,679,806, October 21, 1995.
68. Shen, J.L., Aglucone Isoflavone Enriched Vegetable Protein Fiber, U.S. Patent 5,352,384, October 4, 1994.
69. Waggle, D.H., and B.A. Bryan, Recovery of Isoflavones from Soybean Molasses, U.S. Patent 6,706,292, March 16, 2004.
70. Kelly, G.E., J.L. Huang, M.G. Deacon-Shaw, and M.A. Waring, Preparation of Isoflavones from Legumes, U.S. Patent 6,146,668, November 14, 2000.
71. Iwamura, J., Process for Isolating Saponins and Flavonoids from Leguminous Plants, U.S. Patent 4,428,876, January 31, 1984.
72. Bates, G.A., and B.A. Bryan, Process for Separating and Recovering Protein and Isoflavones from a Plant Material, U.S. Patent 6,703,051, March 9, 2004.
73. Gugger, E., and R. Grabiell, Production of Isoflavone Enriched Fractions from Soy Protein Extracts, U.S. Patent 6,565,912, May 20, 2000.
74. Empie, M., and E. Gugger, Method of Preparing and Using Isoflavones, U.S. Patent 6,261,565, July 17, 2001.
75. Hilaly, A.K., B. Sandage, and J. Soper, Process for Producing High Purity Isoflavones, U.S. Patent Application No. 20040019226 A1, January 29, 2004.
76. Munro, I.C., M. Haywood, J.J. Hlywka, A.M. Stephen, J. Doull, G. Flamm, and H. Adlercredtz, Soy Isoflavones, a Safety Review, *Nutr. Rev.* 61:1–33 (2003).
77. Bennink, M.R., A.S. Om, and Y. Miyagi, Inhibition of Colon Cancer (CC) by Soy Flour but Not by Genistin or a Mixture of Isoflavones [meeting abstract], *FASEB J.* 13:A50–A50 (1999).
78. Keinan-Boker, L., Y.T. van der Schouw, D.E. Grobbee, and P.H.M. Peeters, Dietary Phytoestrogens and Breast Cancer Risk, *Am. J. Clin. Nutr.* 79:282–288 (2004).
79. Allred, C.D., K.F. Allred, Y.H. Ju, T.S. Goeppinger, D.R. Doerge, and W.G. Helferich, Soy Processing Influences Growth of Estrogen-Dependent Breast Cancer Tumors, *Carcinogenesis* 25(7):1–9 (2004).
80. Allred, C.D., K.F. Allred, Y.H. Ju, S.M. Virant, and W.G. Helferich, Soy Diets Containing Varying Amounts of Genistein Stimulate Growth of Estrogen-dependent (MCF-7) Tumors in a Dose-dependent Manner, *Cancer Research* 61:5045–5050 (2001).
81. Hsieh, C.Y., R.C. Santell, S.Z. Haslam, and W.G. Helferich, Estrogenic Effects of Genistein on the Growth of Estrogen Receptor-positive Human Breast Cancer (MCF-7) Cells *in Vitro* and *in Vivo*, *Cancer Res.* 58:3833–3838 (1998).

Chapter 4

Soybean Saponins: Chemistry, Analysis, and Potential Health Effects

Jun Lin and Chunyang Wang

South Dakota State University, Brookings, SD 57006

Saponins, a class of natural surfactants, are sterols or triterpene glycosides that are present naturally in a wide variety of plants. Many different saponins occur naturally, even within a single plant species (1). Saponin-containing plants often display a creamy, even foamy, texture that distinguishes them from other plants. Only about 30 of these plants are regularly consumed by humans, mostly vegetables, legumes, and cereals—ranging from beans to spinach, tomatoes, potatoes, and oats. Legumes such as soybeans and chickpeas are the major sources of saponins in the human diet (1). Sources of non-dietary saponins include alfalfa, sunflower, horse chestnut, and a wide variety of herbs (2). The saponin content of major soybean products is 0.17–6.16% in whole soybeans, 1.8% in soya hulls, 0.35–2.3% in defatted soy flour, and 0.06–1.9% in tofu (discussed later; see [Tables 4.1](#) and [4.2](#)).

Soy saponins are one of the most important sources of dietary saponins, since soybeans are the main protein source in many vegetarian diets. Three groups of soyasaponins have been found: groups A, B, and E (3–6). Soy saponins were historically listed as antinutritional factors (7). Yet recent studies have shown that saponins are potential functional food components because of their physiological properties. These include cholesterol-lowering (8–10), potential cancer preventive (11–13), potential human immunodeficiency virus (HIV) infection inhibitive (14–16), immunomodulating, and antioxidative (17,18) properties. To date, many analytical methods for saponins in plants have been developed. These methods use high-performance liquid chromatography (HPLC), liquid chromatography/mass spectrometry (LC/MS), mass spectrometry (MS), thin-layer chromatography (TLC), nuclear magnetic resonance (NMR), and visible/near-infrared spectroscopy (Vis-NIR). This chapter addresses structure, characteristics, biological activities, and analysis of saponins in soybeans.

Structure and Chemical Characteristics

Saponins are amphiphilic compounds in which hydrophilic sugars (pentoses, hexoses, or uronic acids) are linked to hydrophobic aglycones (the sapogenin) that may be either a sterol or a triterpene. The amphiphilic nature of saponins dominates their physical properties. They are surface active, forming stable foams and acting as emulsifying agents. They generally have a strong hemolytic activity and appear to form micelles in

TABLE 4.1
Saponin Content in Soybeans^a

Soybean	Method	Saponins	Level (%)	Reference
Whole soybean	Modified Liebermann-Burchard reagent	Total	0.578 ^b	Gestener <i>et al.</i> , 1966 (23)
Soybeans	HPLC-ELSD ^c	Total	0.47 (0.530)	Ireland <i>et al.</i> , 1986a (24)
Soybean (whole seed)	HPLC-ELSD ^c	Soyasapogenol A	0.224	Ireland and Dziedzic, 1985 (25)
		Soyasapogenol B	0.246	
		Soyasapogenol	0.183	
		Soyasapogenol C	0.181	
		Soyasapogenol D	N.D.	
Soybean (China)	HPLC-fluorescent coumarin derivation	Soyasapogenol E	0.166	Kitagawa <i>et al.</i> , 1984b (26)
		Soyasaponin A1	0.065 (0.071)	
		Soyasaponin A2	0.032 (0.035)	
		Soyasaponin I	0.157 (0.172)	
		Soyasaponin II+III	0.044 (0.048)	
Soybean (USA)	HPLC-fluorescent coumarin derivation	Total soyasaponins	0.298 (0.326)	Kitagawa <i>et al.</i> , 1984b (26)
		Soyasaponin A1	0.062 (0.068)	
		Soyasaponin A2	0.027 (0.030)	
		Soyasaponin I	0.125 (0.138)	
		Soyasaponin II+III	0.040 (0.044)	
Soybean (Canada)	HPLC-fluorescent coumarin derivation	Total soyasaponins	0.254 (0.280)	Kitagawa <i>et al.</i> , 1984b (26)
		Soyasaponin A1	0.049 (0.055)	
		Soyasaponin A2	0.023 (0.025)	
		Soyasaponin I	0.119 (0.131)	
		Soyasaponin II+III	0.034 (0.037)	
Whole soybeans (IL, USA)	TLC	Total saponins	0.255 (0.247)	Fenwick and Oakenfull, 1981(27) Hu <i>et al.</i> , 2002 (28)
Soybean (USA)	HPLC-UV	Soyasaponin V	0	
		Soyasaponin I	0.0227	
		Soyasaponin II	0.0091	
		Soyasaponin αg	0.0228	
		Soyasaponin βg	0.307	
		Soyasaponin βa	0.623	
		Total soyasaponins	0.424	
Dried navy beans	TLC-densitometry	Total	0.32	Gurfinkel and Rao, 2002 (29)
Dried kidney beans			0.29	
Soybean seed (457 varieties, in and outside of Japan)	HPLC-TLC-UV	Total	0.62–6.16	Shiraiwa <i>et al.</i> , 1991 (4)

^aYields (%) are on an as-is basis. Yields (%) calculated from the dried materials are given in parentheses.

^bYields (%) are on a dry-matter basis.

^cHPLC with evaporative light-scattering detector.

TABLE 4.2

Saponin Content in Soy Products^a

Soy Material	Method	Saponins	Level (%)	Reference
Defatted flour	Modified Liebermann-Burchard reagent	Total	0.483 ^b	Gestener <i>et al.</i> , 1966 (23)
Soybean (defatted flour)	HPLC-ELSD	Soyasapogenol A	0.224	Ireland and Dziedzic, 1985 (24)
		Soyasapogenol B	0.287	
		Soyasapogenol B1	0.147	
		Soyasapogenol C	0.135	
		Soyasapogenol D	N.D.	
		Soyasapogenol E	0.209	Fenwick and Oakenfull, 1981 (27)
Defatted soy flour	TLC	Total saponins	2.258 (2.5)	
Soy hulls			1.806 (2.0)	
Tofu			1.896 (2.1)	
Protein isolate	'Promine-D' ^c		0.272 (0.3)	
	'G.L. 750' ^d		0.727 (0.8)	
	'Maxten C' ^e		1.74 (1.9)	
	'Maxten E' ^f		2.315 (2.5)	
Lecithin	'Vitaplex' ^g		2.749 (2.9)	
	'Crown' ^h		5.009 (5.3)	
Toasted, defatted soy flour (UK)	HPLC-ELSD	Total saponins	0.67 (0.720)	Ireland <i>et al.</i> , 1986a (24)
Full fat, enzyme-active soy flour ⁱ			0.43 (0.468)	Kitagawa <i>et al.</i> , 1984b (26)
Full fat, heat-treated soy flour			0.49 (0.531)	
Soymilk I ^j			0.026 (0.257)	
Soymilk II ^j			0.022 (0.310)	
Tofu (bean curd)	HPLC-fluorescent	Total	0.045 (0.301)	
Yuba (dried bean curd)	coumarin derivation		0.378 (0.407)	Gurfinkel and Rao, 2002 (29)
Miso (bean paste)			0.074 (0.148)	
Defatted soy flour	TLC-densitometry	Total	0.58 ²	
Soybean flour	HPLC-UV	Total	0.346	
Tofu (firm, <i>mori-nu</i>)			0.057	
Soymilk (White Wave, Inc.)			0.046	Hu <i>et al.</i> , 2002 (28)

^aYields (%) are on an as-is basis. Yields (%) calculated from the dried materials are given in parentheses.

^bYields (%) are on a dry-matter basis.

^cSoy protein isolate obtained from Central Soya Co., Inc., Illinois, USA.

^dSoy protein isolate obtained from Griffith Laboratories Pty. Ltd., Victoria, Australia.

^eTextured soy protein obtained from Miles Laboratories (Australia) Pty. Ltd., Victoria, Australia.

^fCrown Vitamins Pty. Ltd., Chatswood, New South Wales.

^gVitaplex Pty Ltd., Chatswood, New South Wales.

^hNV ALPRO Protein Products, Zuidkaai 33, B-8700 Izegem, Belgium.

ⁱUSDA Grade II, British Soya Products, Ware, UK.

^jArdex D. H. V.

much the same way as detergents. These properties are exploited in most of the technological uses of saponins, such as in shampoos and carbonated drinks (1).

Three main types of steroid aglycones are derivatives of spirostan, furostan, and nautigenin (Fig. 4.1). The most well-known triterpene aglycones are derivatives of oleanan (Fig. 4.1). The oleanan aglycone contains one or more hydroxyl groups; in addition, carboxylic groups and double bonds may be present. The sugar compounds are generally attached at the C-3 position of the aglycones (sapogenins). Some sapogenins contain two sugar chains attached at the C-3 and C-22 positions. The saponins that have one sugar chain attached at the C-3 position are called monodesmoside saponins and those that contain two sugar chains are the bidesmoside saponins. Triterpene saponins can be neutral or acidic. Acidity is connected with the presence of uric acids in the sugar chain or a carboxylic group in the sapogenin (17,19).

Galactose, arabinose, rhamnose, glucose, glucuronic acid, and fructose are the most common sugars in saponin structures. The number of monosaccharide units in the sugar chain is between one and eight (19). Five sapogenins have been identified in soybeans (Fig. 4.2). Soybean saponins have been classified into three groups: A, B, and E. Group A saponins are bidesmoside saponins with olean-12-en-3b,21b,22b,24-tetraol (soyasapogenol A) as the aglycone. These aglycones are

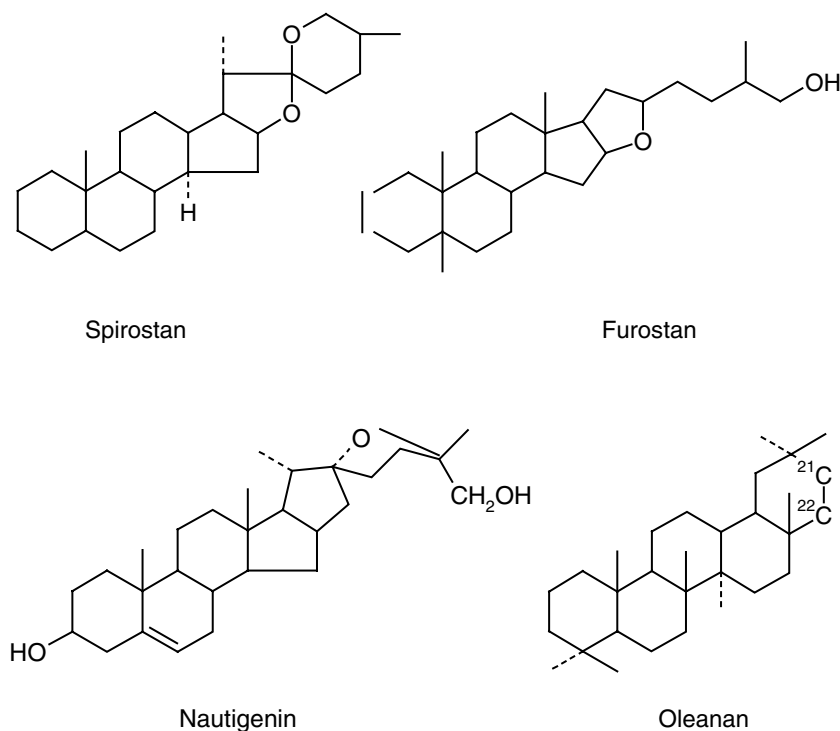
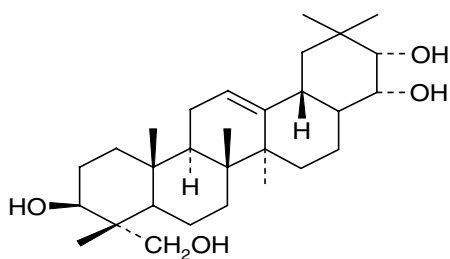
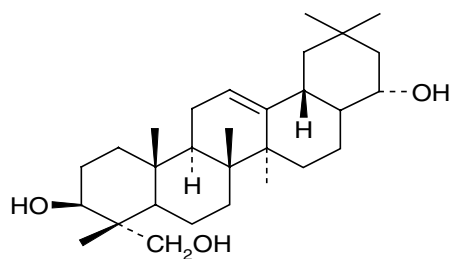


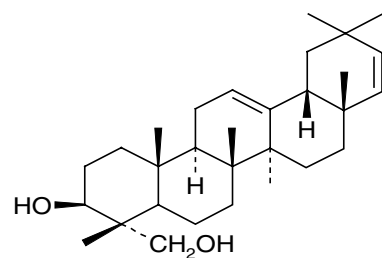
Figure 4.1. Structures of steroid and triterpene aglycones (19).



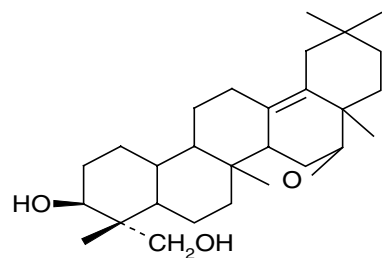
Soyasapogenol A



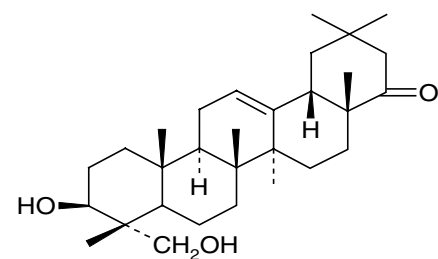
Soyasapogenol B



Soyasapogenol C



Soyasapogenol D



Soyasapogenol E

Figure 4.2. Structures of the five kinds of soyasapogenols (1).

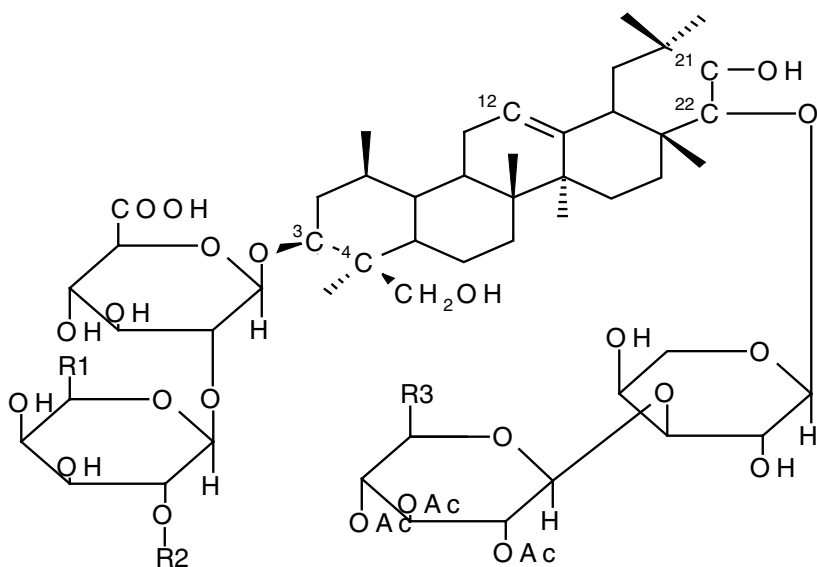
linked to two sugar chains attached to positions 3 and 22. Eight kinds of acetylated, and six kinds of deacetylated, saponins have been identified in this group.

Group A saponins in soybeans were identified by Okubo *et al.* (15) and Kitagawa *et al.* (20–22). They have two different naming systems. Okubo's group named them soyasaponin Aa, Ab, Ac, Ad, Ae, Af, Ag, and Ah according to their elution sequence from chromatography (3). Kitagawa's group only found six of these. They did not find soyasaponins Ac and Ad. They named the rest of them as soyasaponin A4, A1, A5, A2, A6, and A3, respectively (22). The structures and the naming systems of group A soyasaponins are shown in [Figure 4.3](#).

Group B and E saponins are monodesmoside saponins with olean-12-en-3 β ,22 β ,24-triol (soyasapogenol B) and olean-12-en-3 β ,24-diol-22-one (soyasapogenol E) as their aglycones. Group B soyasaponins contain only one ether-linked sugar chain, attached to position 3. There are also two naming systems for group B soyasaponins. Kitagawa *et al.* (20) used soyasaponin I, II, III, IV, and V. Okubo *et al.* (15) used soyasaponin, Bb, Bc, Bb', Bc', and Ba. The differences among these five B-group soyasaponins lies in the sugar composition of the oligosaccharide chain at C-3. Kudou *et al.* (24) reinvestigated the composition and the structures of the native group B soyasaponins in soybean seeds and isolated five kinds of saponins, which they named soyasaponins α g, β g, β a, γ g, and γ a, according to elution order from HPLC. The structures were characterized as having a 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) moiety attached via an ether linkage to the C-22 hydroxyl of soyasaponins Ba, Bb, Bc, Bb', and Bc'. DDMP provided these saponins with UV absorption properties at 292 nm (24). DDMP saponins were detected as major saponin constituents when much milder extracting conditions were used. Group E soyasaponins are named soyasaponin Be and Bd (4). The structures of group B, E, and DDMP saponins are shown in [Figure 4.4](#).

Natural Occurrence and Effects of Processing

Composition and content of saponins in soybeans of different variety, cultivation year, and maturity have been investigated in many studies ([Table 4.1](#)). Shiraiwa *et al.* (4) studied the content of group A, B, and E saponins in seed hypocotyls of 457 varieties of soybeans cultivated in and outside Japan from 1985 to 1988. They found that the saponin composition in soybean seed was not affected by the year of cultivation, but was dependent on variety. There were no remarkable differences among varieties in regard to the composition of group B and group E saponins compared with group A saponins. They also found that the saponin composition and content in soybean seed was affected by the degree of maturity. For group B saponins, the I and II isomers were the main constituents. The content of group B saponins decreased with seed maturation, and this group of saponins was absent in mature seed hypocotyls. In the seed harvested at different degrees of maturity, the seed in the early stage of maturity contained numerous group A saponins—Aa, Ab, Ac, Ad, Ae, and Af. As the maturity of the seed progressed, the number of constituents tended to decrease. The content of both group A and group B saponins in seed hypocotyls of

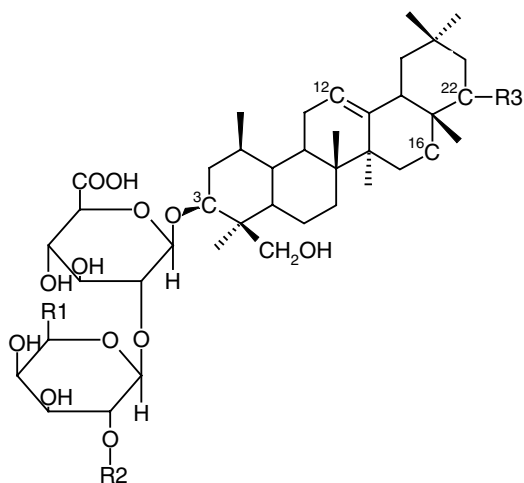


	R ₁	R ₂	R ₃
Soyasaponin Aa (A4)	CH ₂ OH	β-D-Glc	H
Soyasaponin Ab (A1)	CH ₂ OH	β-D-Glc	CH ₂ Oac
Soyasaponin Ac	CH ₂ OH	α-L-Rha	CH ₂ Oac
Soyasaponin Ad	H	β-D-Glc	CH ₂ Oac
Soyasaponin Ae (A5)	CH ₂ OH	H	H
Soyasaponin Af (A2)	CH ₂ OH	H	CH ₂ Oac
Soyasaponin Ag (A6)	H	H	H
Soyasaponin Ah (A3)	H	H	CH ₂ Oac

Figure 4.3. Structures of group A saponins (23).

soybeans harvested in Japan decreased from October 13 to December 1 and increased from December 1 to December 14 during 1988. Tsukamoto *et al.* (6) investigated the effect of different temperatures during seed development on the content of DDMP-conjugated saponins and found that the range of temperatures studied did not have any significant effect on the DDMP-conjugated saponin content.

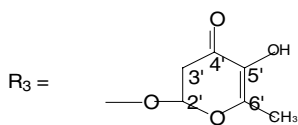
Recently, Rupasinghe *et al.* (31) studied soyasapogenol A and B distribution in soybean in relation to seed physiology, genetic variability, and growing location.



Group B saponin $R_3 = \text{OH}$

Group E saponin $R_3 = \text{O}$

DDMP saponin



	Group B	Group E	DDMP	R_1	R_2
Soyasaponin	Ba (V)	Bd	αg	CH_2OH	$\beta\text{-D-Glucosyl}$
Soyasaponin	Bb (I)	Be	βg	CH_2OH	$\alpha\text{-L-Rhamnosyl}$
Soyasaponin	Bc (II)		βa	H	$\alpha\text{-L-Rhamnosyl}$
Soyasaponin	Bb_ (III)		γg	CH_2OH	H
Soyasaponin	Bc_ (IV)		γa	H	H

Figure 4.4. Structures of group B, E, and DDMP saponins (23).

They found that seed germination had no influence on soyasapogenol A content but increased the accumulation of soyasapogenol B. Soyasapogenols were mainly maintained in the axis of the seeds as compared with the cotyledons and seed coat. Ten food-grade soybean cultivars grown in four locations of Ontario, Canada, were used in their study. They observed a significant variation in soyasapogenol content among

cultivars and growing location. They also mentioned that there were no significant correlations between the content of soyasapogenols and the total aglycones among 10 cultivars grown in four locations. Hu *et al.* (29) had similar results in 46 cultivars of soybean grown in Iowa. But Rupasinghe *et al.* (31) thought that this relationship needed to be further analyzed using a larger number of more genetically diverse soybean cultivars. Soy products contain different amounts of soyasaponins (Table 4.2). However, similar products were shown to have dramatically different concentrations by different laboratories. This supports the urgent need for interlaboratory studies and the development of a uniform method.

DDMP-conjugated soyasaponins (α g, β g, β a, γ g, and γ a) can be converted to soyasaponin I, II, III, IV, and V, respectively, when they lose DDMP. It has been shown that heating or prolonged extraction and storage after harvesting release soyasaponin I from the DDMP-conjugated form, which could be due to natural enzymatic processes in the cotyledon (32). Hu *et al.* (29) studied saponin concentrations of various soy products. The effects of processing can be seen in their results. The DDMP-conjugated soyasaponins were the major components in the raw soybean flour, while the non-DDMP soyasaponins were the major forms in the processed soy products. High concentrations of soyasaponins α g and β g and their non-DDMP forms V and I were found in the toasted soy hypocotyls. The group B soyasaponins were undetectable in ethanol-washed soy protein concentrates but were present in acid-washed soy protein concentrates and soy protein isolates. Soymilk, tempeh, and tofu appeared to be low in soyasaponin content compared to the raw soybean on “as-is” bases. However, the soyasaponin concentrations on a dry basis in these soyfoods are close to or greater than those in the raw soybean flour.

Biological and Nutritional Properties of Saponins

The biological activities of saponins are closely related to their chemical properties. Saponins might be considered as functional food components because of their potential health benefits. These include cholesterol-lowering (8–10), potential cancer preventive (11–13), potential human immunodeficiency virus (HIV) infection inhibitive (14–16), immune-modulating, and antioxidative (17,18) properties. Biological activities of saponins are diverse and depend on the source and the type of saponins.

Cholesterol-Lowering Properties and Reduction of Heart Disease Risk

Cardiovascular disease (CVD) is a general term for heart and blood vessel diseases. These include high blood pressure, coronary heart disease (CHD), stroke, and rheumatic heart disease. One-half of CVD-related deaths are due to CHD. The main causes of CVD are atherosclerosis (buildup of fatty deposits in the inner lining of the blood vessels) and thrombosis (blood clots formed by clumped platelets that block blood vessels). High levels of low-density lipoprotein (LDL) cholesterol, especially oxidized LDL, lead to atherosclerosis (33).

Animal and Human Studies. Isolated saponins and foods containing saponins have been shown to lower plasma cholesterol in a number of animal species (34). Oakenfull *et al.* (35) found that dietary saponins lowered plasma and liver cholesterol in rats on a high-cholesterol diet and lowered liver cholesterol in rats on a low-cholesterol diet. Dietary saponins were found to increase the excretion of bile acids and neutral sterols in the feces. With a high-cholesterol diet saponins increased the rate of bile acid secretion. Therefore it has been suggested that foods containing saponins could be important in formulating hypocholesteremic diets for human consumption (8,36). The saponin fractions from garlic were found to lower plasma total and LDL cholesterol without changing high-density lipoprotein (HDL) cholesterol levels in a hypercholesterolemic animal model. Several steroid saponins occur in both garlic and aged garlic extract (10).

Mechanism of the Hypocholesterolemic Activity of Saponins. Saponins and bile acids are both amphiphilic compounds. In aqueous solution, they form small micelles individually. Their hydrophobic triterpene or steroid groups stack together like small piles of coins. The hydrophobic groups of the two types of compounds interweave with each other. The stereo and electrostatic constraints to the formation of micelles are relieved and the stacks become greatly extended, incorporating many hundreds of molecules (Fig. 4.5).

Bile acids are absorbed through the wall of the small intestine by passive diffusion and active transport. Passive absorption takes place along the entire length of the

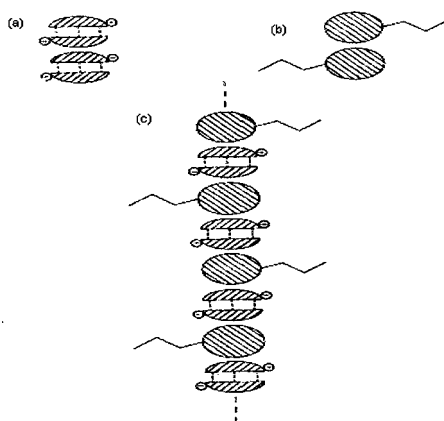


Figure 4.5. Schematic diagram of the structures of the micelles formed by (a) bile acids, (b) saponins, and (c) saponins plus bile acids. The hydrophobic triterpene group of the saponin is indicated by an ellipse; each monosaccharide group is indicated by a straight line (19).

ileum and jejunum; active transport is confined to the terminal ileum. Saponins can interact with cell membranes, as is obvious from their hemolytic activity (37). Electron microscopy has revealed that saponins can permeabilize plasma membranes, releasing soluble proteins while preserving many cytoplasmic membranes (38). Nuclear magnetic resonance studies have shown that the formation of the immobilized complex saponins—cholesterol in the membranes might be related to the hemolytic activity (39). The effects of saponins on both passive absorption and active transport can be explained as simply due to the reduction in the concentration of free (as opposed to micellar) bile acids. Low concentration of free bile acids seems to lower the efficiency of lipid absorption (35) and presumably also affects absorption of fat-soluble vitamins. Another factor to be considered arises from another observation by West *et al.* (40) that casein given to rabbits loses its hypercholesterolemic effect by the replacement of half of the casein by soy isolate. Proteins that are not completely digested interfere with the absorption of bile acids and may interrupt the enterohepatic circulation of bile acids, which in turn may result in an enhanced loss of steroids in the feces and consequently in lower levels of serum cholesterol. This would imply that soy protein is less digestible than casein, at least in the distal part of the small intestine where the absorption of bile acids takes place. In the study, West *et al.* (40) also mentioned that the maximum extent of digestion of soy protein occurs more distally in the gastrointestinal tract compared to that of casein. This work supported the idea that differences in the digestion of protein, at least at specific sites in the intestine and not necessarily in the overall digestion (i.e., mouth-to-anus digestion), affects the level of cholesterol in the serum.

Formation of mixed micelles in the small intestine by certain saponins and bile acids provides a molecular explanation for the effects of saponins on bile acid and cholesterol metabolism. Micellar bile acid molecules are not available for reabsorption and are thus diverted from the enterohepatic cycle. Consequently ingestion of foods containing saponins would increase fecal excretion of bile acids and lower plasma cholesterol in hypercholesterolemic subjects.

Hypocholesterolemic effects of soybean saponins have been demonstrated by several studies. Isolated soybean saponins reduced diet-induced hypercholesterolemia through an increase in bile acid excretion (41). They also form micelles with bile acids and reduce their absorption *in vitro* (42).

Another potential mechanism for the hypocholesterolemic effect of saponins is their interaction with proteins. Saponins have been shown to interact with proteins and lower their digestibility. This leads to lower absorption of dietary proteins and thus lower caloric intake. Soybean saponins interact with bovine serum albumin (BSA) and decrease the sensitivities against chymotrypsin hydrolysis. BSA became thermally more stable by interacting with saponins (9,43). In a recent study (44), the effects of a saponin fraction on chymotryptic hydrolysis of acid precipitated soybean protein with glycinin and β -conglycinin fractions were examined. Endogenous saponin affected the chymotryptic hydrolysis of soybean protein. Further addition of saponin suppressed the hydrolysis of soybean protein fractions. The effect of saponin on the chymotryptic hydrolysis of glycinin was greater than on that of β -conglycinin. Glycinin acidic

polypeptides and β -conglycinin β -subunit became more resistant to chymotryptic hydrolysis by the addition of saponin.

However, it has been shown that soybean saponin affects the tryptic and chymotryptic hydrolyses of whey protein differently. β -Lactoglobulin and α -lactalbumin became more sensitive to both trypsin and chymotrypsin by interacting with saponin in contrast to serum albumin. Soybean saponin was shown to have different effects on various proteins. Milk whey, which is produced in cheese processing, mainly contains the whey proteins β -lactoglobulin (β -Lg) and α -lactalbumin (α -La), as well as lactose. The hydrolysis level of calcium-depleted α -La that contained saponin was slightly higher than that containing no saponin practically throughout the incubation period. Saponins decreased the chymotryptic and/or tryptic hydrolyses of BSA and the soybean globulin fraction. These decreases were thought to be the result of the conformational change in the proteins caused by interaction with saponin covering target residues of the proteases. However, the whey proteins became sensitive to trypsin and chymotrypsin by interacting with saponin. The conformational changes induced by interaction with saponin made some groups of the protein molecular structure compact and others loose. The effect of saponin was different with each protein, reflecting their individual natures and high-order structures (45).

Cancer Prevention

Epidemiological Evidence. Epidemiological studies have indicated that diets high in animal fat and low in plant foods are positively correlated with the occurrence of colon and breast cancer, the most common forms of cancer in developed countries (46,47). On the basis of these epidemiological and other experimental studies (48), dietary guidelines recommended increased consumption of vegetables, cereals, legumes, and fruit and decreased intake of fat (49,50). Legumes, especially soyfoods, are major components of these types of diets.

Of the estimated 5.2 million deaths from cancer in 1990, 55% occurred in developing countries. It is also a major cause of death in Western countries. Epidemiological and etiological studies demonstrate that there is a dramatic difference in the risk of certain cancers, including breast and prostate cancers, between populations of the Western countries and those of the Eastern countries. Death rates from cancer in men and women from various countries are shown in [Figure 4.6](#). In Japan, the average total consumption of soybeans, soy products, and pulses are estimated to be 18.0, 14.2, and 8.0 g/d, respectively. On the other hand, total pulse consumption, including soybeans, in some Western countries is estimated at 3–10 g/d (52). The lower incidence of cancer and higher intake of soybeans of Japanese living in Japan compared with those who emigrated to the West (53) suggests that saponins may play an important role in cancer prevention.

According to Dr. Paxton's study (47), the breast cancer rate in the United States is four times that in Japan, five times that in China, and ten times that in Korea. One in nine American women will get breast cancer. The prostate cancer rate in the

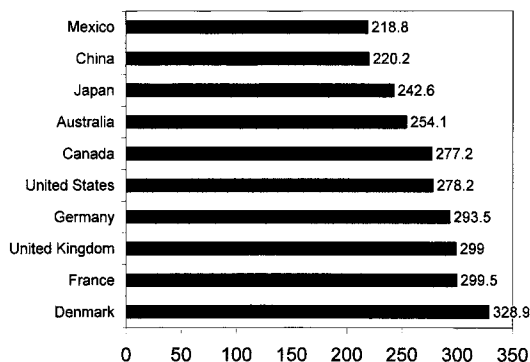


Figure 4.6. Death rates from cancer worldwide (51). Deaths per year, per 1,000,000 population (2000).

United States is five times that of Japan, thirty times that of China and six times that of Korea. One in eleven American men will get prostate cancer. The consumption of saponins in a typical Western diet was about 345 mg per day, while that of a typical Eastern diet was about 1,725 mg per day (47). Although many other factors contribute to these differences in the cancer rates among the populations, saponins are at least partially responsible.

Evaluating data from populations that eat greater quantities of plant-based foods, it was found that the groups consuming foods richest in saponins have lower incidences of breast, prostate, and colon cancer (17).

Animal and Cell Culture Studies. Saponins have direct cytotoxic and growth inhibitory effects on tumor cells. There have been several *in vitro* and *in vivo* studies that have evaluated the cytotoxic effect of saponins on tumor development. The active components in several herbal medicines that have been used as chemotherapeutic agents in Eastern countries were saponins. The extracts of Yunnan Bai Yao, a Chinese herbal drug that contains the saponin formosanin-C, exhibited cytotoxic activity in several cancer cell lines when a tissues culture screen was used (54). Saponins extracted from *Agave cantala* and *Asparagus curillus* significantly inhibited the growth of human cervical carcinoma (JCT-26) *in vivo*, and p 388 leukemia cells *in vitro* (55).

Saponins may also act to delay the initiation and progression of cancers through indirect effects. The interactions between saponins and bile acids are important in cancer prevention. *In vitro*, saponins were shown to form large mixed micelles (1×10^8 Da) with bile acids (42). Similar interactions *in vivo* would reduce the free form of bile acids in the upper gastrointestinal tract and decrease the absorption of bile acids across the mucosa as well as the formation of secondary bile products from primary bile acids. Increases in the fecal excretion of steroids, especially bile acids, were observed after feeding mice semi-synthetic diets containing 1% soybean saponins (42). A similar increase in fecal biliary excretion was observed in mice

ingesting diets containing alfalfa seeds (56). These results suggest that saponins from different dietary sources reduce the availability of bile acids for formation of secondary bile acids by intestinal microflora, and therefore may prevent the development of colon cancer.

During the neoplastic process of colonic epithelial cells, major zones of DNA synthesis for cell proliferation are extended from the normal crypt (57). On the basis of the hypothesis that abnormal proliferation of crypt cells induced by bile acids is either delayed or normalized by saponins that bind to bile acids, mice were fed a diet containing cholic acid with and without Quillaja saponin. In mice fed cholic acid alone, colonic epithelial cell proliferation was increased and the major zone of proliferation was extended. However, colonic epithelial cells of the mice fed diets containing cholic acid and 1% Quillaja saponin showed normal cell proliferative characteristics. Also, the abnormal cell proliferation induced by carcinogen treatment was normalized within 7 weeks of feeding diets containing Quillaja saponin to mice (58).

Sialyltransferases (STs) are a family of glycosyltransferases that catalyze the transfer of sialic acid from cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) to nonreducing terminal positions on the sugar chains of glycoconjugates (glycoproteins and glycolipids). Many studies have demonstrated that hypersialylation, which occurs during certain pathological processes, such as oncogenic transformation, tumor metastasis, and invasion, is associated with enhanced ST activity. Soyasaponin I has been determined to be the most potent and specific ST inhibitor among 7,500 samples including microbial extracts and natural products (59).

The mixture of triterpenoid saponins obtained from an Australian desert tree (Leguminosae) *Acacia victoriae* (Benth) and avicins that contain an acid core with two acyclic monoterpene units connected by a quinovose sugar induce apoptosis in the Jurkat human T cell line by affecting the mitochondrial function (60). Soybean saponins inhibit the formation of DNA adducts, which is the most important reaction of carcinogens with cellular macromolecules initiating carcinogenesis, in human colon and liver cells (12). This study showed that soybean saponins inhibit the growth of human colon carcinoma cells with low toxicity and decreased the ornithine decarboxylase activity that is directly related to cancer cell proliferation. These results indicate that soybean saponins are important modulators in the promotion stage of carcinogenesis. Soybean saponins also repressed 2-acetoxyacetylaminofluorene (2AAAF)-induced DNA damage in a Chinese hamster's ovary (CHO) cells as measured by single-cell gel electrophoresis (alkaline Comet Assay) (61).

Dietary intake of saponins isolated from soy flour significantly reduced the incidence of aberrant crypt foci (ACF) induced by azoxymethane (AOM) in the colonic wall of Carworth Farms (CFI) mice (62). The results showed that soybean saponins at concentrations of 150–600 ppm had a dose-dependent growth inhibitory effect on human carcinoma cells (HCT-15). Viability of these cells was also significantly reduced. Soybean saponins did not increase cell membrane permeability in a

dose-dependent fashion, whereas gypsophilla saponin, a non-dietary saponin, increased permeability with increasing concentrations. Electron microscopy indicated that soybean and gypsophilla saponins alter cell morphology and interact with cell membranes in different ways (17). Also, soybean saponins significantly suppressed colon cancer cell (HT-29) growth in a dose-dependent manner. They inhibited the 12-*O*-tetradecanoyl phorbol 13-acetate (TPA)-stimulated protein kinase C (PKC) activity as defined by the substrate phosphorylation and also effectively induced differentiation. Examination by transmission electron microscopy indicated that soybean saponins induced deformations in plasma and nuclear membranes without abrupt membrane rupture. Results from this study showed that soybean saponin pretreatment significantly reduced the TPA-stimulated total PKC activity dose-dependently. They imply that saponin-membrane interactions possibly affect PKC translocation and directly interfere with the activation of the enzyme (13).

The proposed mechanisms of the anticarcinogenic properties of saponins include direct cytotoxicity, bile acid binding, and normalization of carcinogen-induced cell proliferation. Another potential mechanism involves immune-modulatory effects.

Antiviral Activity

Since the identification of HIV as the causative agent of acquired immune deficiency syndrome (AIDS), it has been reported that some compounds, such as nucleoside analogues, may be useful in the prevention and treatment of AIDS and its related disorder, AIDS-related complex (ARC). Saponins have been shown to affect HIV in vitro using an HTLV-1-carrying cell line, MT-4, and MOLT-4 cell system (14,15). Major work was done on saponins other than soyasaponins. It was found that formosanin-C increased natural killer cells (63) and ginsenosides increased immune response (64).

In general, it is difficult to separate the anticarcinogenic effects of saponins from their immune-modulatory effects. A digitonin saponin, formosanin-C, extracted from Liliaceae and also a component of Yunnan Bai Yao, has been shown to have antitumor activity that acts by modifying the immune system (63). Formosanin-C injected intraperitoneally inhibited the growth of hepatoma cells implanted in C3H/HeN mice. Blood samples from these animals showed that the activity of natural killer cells and the production of interferon were significantly increased. The ginsenoside R_{g1} from the root of *Panax ginseng* was shown to increase both humoral and cell-mediated immune responses (64). Spleen cells recovered from ginsenoside-treated mice injected with sheep red cells as the antigen showed significantly higher plaque-forming response and hemagglutinating antibody titer to sheep red cell antigen. Also, R_{g1} increased the number of antigen-reactive T helper cells and T lymphocytes. There was also a significant increase in natural killer cell activity and lymph node size. Therefore, saponins seem to induce a series of immune responses rather than a single specific response.

Oleanan-type triterpenoidal saponins have anti-herpes simplex virus type 1 (HSV-1) activity. Among sophoradiol glycosides, the order of potency was kailasaponins III > kailasaponins I >> sophoradiol monoglucuronide. Among the trisaccharide group of soyasapogenol B, the order of activity was azukisaponin V > soyasaponin II > astragaloside VIII >> soyasaponin I. In comparison with the activity for a group having the same trisaccharide, the potency of the sapogenol moieties was soyasapogenol E > sophoradiol >> soyasapogenol B. Hence, the carbonyl group at C-22 would be more effective than the hydroxyl group in anti-HSV-1 activity while the hydroxyl group at C-24 could reduce the activity (65).

Soybean saponins isolated from soybean seeds also have inhibitory activity against HIV infection using an HTLV-I-carrying cell line, MT-4. Soyasaponin BI has been shown to completely inhibit HIV-induced cytopathic effects and virus-specific antigen expression 6 days after infection at concentrations greater than 0.25 and 0.5 mg/ml, respectively (14). However, neither soyasaponin BI nor BII had any direct effect on HIV reverse transcriptase activity. Soyasaponin BI also inhibited HIV-induced cell fusion in the MOLT-4 cell system, and virus-specific antigen expression 6 days after infection at concentration greater than 0.25 mg/ml (15). These authors attribute the inhibitory effects of soyasaponin BI to the preventable effects of HIV-induced cell fusion, because it is clear that soyasaponin BI had no effect on HIV reverse transcriptase activity (23).

Hayashi *et al.* (16) studied the antiviral activities of two saponins, soyasaponins I and II, isolated from soybean. The viruses in the studies included HSV-1, human cytomegalovirus (HCMV), poliovirus, influenza virus, and HIV-1. The results are shown in Table 4.3. ACV (acyclovir) and GCV (ganciclovir) were used as positive controls for anti-HSV-1 and anti-HCMV assays, respectively. Soyasaponin II showed more potent inhibition against those viruses. However, no inhibiting activity was found against poliovirus. HSV-1 was the virus most susceptible to soyasaponin II among the viruses tested. Soyasaponin I contains galactose in its oligosaccharide moiety, whereas soyasaponin II has arabinose residue. These differences of structure might reflect the difference of cytotoxic activity between soyasaponins I and II.

Antinutritional Properties

Saponins have long been known to cause lysis of erythrocytes when given in vitro. The hemolytic activity of saponins has been extensively used as a means of detecting and “quantifying” saponins in plant material. The hemolytic activity of soyasapogenols may be low, because soyasapogenols are nonpolar molecules. The effect of soybean saponins on the growth of chicks, mice, rats, and *Tribolium castaneum* larvae and on the survival time of tadpoles and guppies are different (66). Soybean saponins did not impair the growth of chicks, rats, and mice. They caused slight growth retardation of *Tribolium castaneum* larvae. Soybean saponins showed a detrimental effect on tadpoles and guppies (Table 4.4). Saponins administered orally to mammals seem to have no toxic effects (8).

TABLE 4.3Effect of Soyasaponin II on the Cell Growth and Replication of Virus^a

Drug	Virus	Host Cell	Cytotoxicity (CC ₅₀ ^b , mM)	Antiviral Activity (IC ₅₀ ^c , mM)	Selectivity Index (CC ₅₀ /IC ₅₀)
Soyasaponin II	HSV-1	HeLa	1,703 ± 78	54 ± 5.4	32 ± 4.7
	HCMV	HEL	1,650 ± 264	104 ± 13	16 ± 2.4
	Poliovirus	Vero	1,620 ± 140	>1000	<2
	Influenza virus	MDCK ^d	1,300 ± 164	88 ± 13	15 ± 0.73
	HIV-1	MT-4	1,270 ± 62	112 ± 11	11 ± 1.1
Acyclovir	HSV-1	HeLa	4,910 ± 271	4.8 ± 0.70	1,031 ± 106
Ganciclovir	HCMV	HEL ^e	2,010 ± 107	1.5 ± 0.21	1,333 ± 186

^aEach value is the mean ± standard deviation of triplicate assays (15).^bCC₅₀: the 50% inhibitory concentration obtained using host cells.^cIC₅₀: the 50% inhibitory concentration against virus.^dMDCK: Madin–Darby canine kidney.^eHEL: Human embryonic lung.**TABLE 4.4**Effect of SBSE (Soybean Saponin Extract) on the Longevity of Tadpoles (*Bufo viridis*) and Guppies (*Lebistes reticulatus*)^a

SBSE in Medium %	Average Lifetime (min)	
	Tadpoles	Guppies
0.10	44	41
0.20		23
0.25	25	
0.40		16
0.50	13	

^aData from Ishaaya *et al.* (64).

Other Health Implications

Antioxidant Activity. Soyasaponins have antioxidant activity. Tsujino *et al.* (67) reported that the antioxidant activity of chromosaponin I (CS I, soyasaponin βg), the natural form of soyasaponin I, is comparable with that of urate. The study showed that soyasaponin βg inhibited the oxidation of phosphatidylcholine liposomal membranes induced by a water-soluble radical initiator, 2,2'-azobis-(2-amidinopropane) dihydrochloride. DDMP contributes to the saponin's antioxidant activity. Soyasaponin I exerted no antioxidant activity. In a study by Yoshikoshi *et al.* (68), however, soyasaponins βg and I were shown to inhibit hydrogen peroxide damage to mouse fibroblast cells. They concluded that water-soluble soybean saponins protected the cell from damage by hydrogen peroxide.

Furthermore, group A and group B saponins also have antioxidant activity, hepatoprotective effects, and emulsification properties (18,23).

Hemolytic Activity. Adjuvants have been developed widely for potential immunological and biological applications. Many good adjuvant components derived from both artificial and natural products are available. They include aluminum salts (69), oil-based adjuvants (70), nonionic block copolymers (71), muramyl dipeptides (72), carbohydrate polymers (73), and saponins (74). Some adjuvant saponins have hemolytic activity (75). However, soyasaponins and lablabosides in adjuvants showed little hemolytic activity (76).

Gestetner *et al.* (77) found that neither soybean saponins nor soybean sapogenins could be found in the blood of lab animals. Ingested soybean saponins were hydrolyzed into sapogenins and sugars by the cecal microflora of chicks, rats, and mice. Saponin-hydrolyzing enzymes from the cecal microflora of rats were partially purified by successive column chromatography on DEAE-cellulose and calcium phosphate (hydroxyl apatite) in the presence of 2-mercaptoethanol. The *in vitro* hemolytic activity of soybean saponins on red blood cells was fully inhibited in the presence of plasma or its constituents.

Hepatoprotective Activity. Ohminami *et al.* (78) found that the administration of total soyasaponins in a high-fat diet containing peroxidized corn oil could reduce slight hyperlipidemia and reduce the levels of serum lipids—total cholesterol (TC), triglyceride (TG), and free fatty acids (FFA)—in rats. Oral administration of soyasaponins also prevented increases in serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) that were derived from liver injury caused by peroxide and FFA in rats on a high-fat diet. Saponins were shown to prevent liver injury and hyperlipidemia. Soyasaponins I, II, III, A1, and A2 inhibited heat-mediated chemical peroxidation of corn oil. Two possible mechanisms were proposed for the protective actions of soyasaponins against liver injury (78). One was that soyasaponins inhibited the production of lipid peroxide both *in vitro* and *in vivo*. The other was that the soyasaponins inhibit the destructive action of lipid peroxide on hepatocytes. A similar result was also found by Sung and Park (18). In their study, soybean saponins were shown to inhibit the cell growth, cellular lipid peroxidation, and antioxidative enzyme activities of Hep G2 cells. Malondialdehyde content was significantly reduced by saponin (72%). Soybean saponins significantly increased cellular superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione S-transferase (GST).

The hepatoprotective effects of soyasapogenols A and B were investigated by Sasaki *et al.* (79) and Kinjo *et al.* (80). Kinjo *et al.* determined the hepatoprotective actions of soyasaponins I–IV, which have soyasapogenol B as their aglycone, toward immunologically induced liver injury on primary cultured rat hepatocytes. The action of soyasaponin II was almost comparable with that of soyasaponin I, whereas soyasaponins III and IV were more effective than soyasaponins I and II. This means that the disaccharide group shows greater protective effect than the trisaccharide group. Furthermore, the saponins having a hexosyl unit show a slightly greater protective effect than that of the pentosyl unit in each disaccharide group or trisaccharide group. Structure and activity relationships suggest that the sugar moiety linked at C-3 might play an important role in hepatoprotective actions of soybean saponins.

Derivatization of soyasapogenol A and the hepatoprotective activities of the derivatives were studied by Sasaki *et al.* (79). Fifteen derivatives of soyasapogenol A were tested. Hepatoprotective effects of soyasapogenol A derivatives have been evaluated in aflatoxin B1-induced Hep G2 cells, and it has been found that most of them showed improved activities compared to the parent soyasapogenol and that morphological changes in the cultured Hep G2 cells treated with hepatoprotective compounds were significantly less than those in the cells treated with soyasapogenol B.

Anti-obesity Action. Yoshiyuki and Okuda (81) designed two animal models to study obesity. They were gold thioglucose (GTG)-induced obesity and high-fat diet-induced obesity in mice. They found that mice with GTG-induced obesity displayed hyperinsulinemia, high sucrase activity of the intestinal mucosa, and enlarged surface area of villi of the upper small intestine associated with an increase of food consumption. From their experiments, they discovered that oral administration of total soyasaponins prevented development of obesity and an increase of the serum insulin level in GTG-treated mice. Total soyasaponins also reduced the enlargement of the absorptive surface area of the upper small intestine and the increase of parametrial adipose tissue weight. Therefore, soyasaponins may be effective in preventing development of obesity.

Isolation and Measurement of Saponins in Soybean

Detection and Isolation of Saponins in Soybean

The presence of saponins is readily indicated by their hemolytic activity and their ability to form stable foams in aqueous solutions. These properties are characteristic of surfactants in general and are not unequivocal evidence for the presence of saponins; they are good indications that saponins might be present, but other methods are required for more a definite identification (1).

Saponins can be isolated from plant materials by extraction with organic solvents. The plant material is first extracted with acetone or diethyl ether, preferably using a Soxhlet extractor to remove lipids and pigments. The solvent is then changed to methanol to give a crude extract containing the saponins (1). More recently, much milder extraction conditions were used to determine the natural state of saponins in plant samples. It is possible to demonstrate the presence of saponins in the crude extract by several instrumental methods, including TLC, HPLC-MS, GC-MS, and others, without further purification steps.

To date, many methods for the determination of saponin content in plants have been developed. Most of them focus on using HPLC, LC/MS, MS, TLC, NMR, and Vis-NIR spectroscopic methods. Saponins can be isolated from plant materials by extraction with organic solvents. There are two general ways of quantifying saponins after extraction. The first one usually involved hydrolysis of the plant extracts, followed by titrimetric (82), GC (83), or HPLC (25,27,84,85) determination of the released aglycones. The second approach was to measure saponins directly. Direct

saponin measurement can be achieved by HPLC with UV detection, although saponins must be derivatized post- or pre-column because of their poor UV absorbance (86–89). Recently, evaporative light-scattering detection (ELSD) was utilized. With ELSD, no derivatization was needed for HPLC determination of saponin content (25,90–93). Both normal and reverse-phase HPLC systems have been used. Other authors have focused on using MS/NMR (94), HPLC/MS (95–97), LC/MS/MS (98,99), and Vis-NIR (100) to determine saponins after extraction and centrifugation.

Many methods have also been developed to isolate and quantify saponins in soybeans since the 1970s. Most of them utilized chromatographic methods. They include TLC followed by densitometry (28,30), GC after derivatization (20), HPLC using UV detection (29,101). Ireland and Dziedzic (27) used HPLC to quantify the sapogenins (aglycones) released after hydrolysis of the saponins. Wolf and Thomas (102) evaluated 22 solvent systems for TLC of soybean saponins on silica gel. They found that a maximum of four fractions were separated by single development with different solvents, and that six successive developments with chloroform-methanol-water (65:25:4) separated soybean saponins into 10 or more fractions. Kitagawa *et al.* (21) used fluorescent coumarin derivatives of saponins in their HPLC method. Both of these groups obtained sapogenin profiles and content after hydrolysis of saponins. They were able to estimate saponin content by using the sapogenin/carbohydrate ratio. In the HPLC method by Kitagawa *et al.* (21), the use of fluorescent coumarin derivatives overcame difficulties in detecting soyasaponins. This method was unable to provide information on the proportion of acetylated or free saponins. The method didn't separate the coumarin derivatives of soyasaponins II and III. The fluorescent coumarin derivatives are formed by esterification with the carboxylic acid moiety of the glucuronic acid residue common to all five types of soyasaponins. Therefore, it is not possible to develop and extend this method to the analysis of neutral saponins. Although these methods may be useful in providing structural information, they are less useful for quantitative analysis due to the potential loss of materials during hydrolysis and derivatization. Recently, there have been several attempts to overcome the detection problems. These include detection of the underivatized saponins at 190–210 nm (103,104) and monitoring with an ELSD (26). But recently it has been found that some saponins contain a DDMP moiety attached via an ether linkage to the C-22 hydroxyl of group B and E soyasaponins (24). Detection and measurement of DDMP saponins by HPLC is easier than that of group A, B, and E saponins due to their absorbance at 292 nm. Few studies have been done using HPLC-ELSD, with mild extraction conditions.

Quantitative Determination of Saponins from Soybean

Tables 4.1 and 4.2 show a variety of analytical methods that have been used by different authors and their effectiveness. The first quantitative method was developed by Birk *et al.* (105). The method determined saponin content after a purification procedure. A useful indication of saponin content from a sample of plant materials—

albeit only a lower limit—can be obtained simply by determining the yield of purified saponin, following the procedure of Birk *et al.* (105). Alternatively, other methods were used to quantify saponins by utilizing their properties, such as a foam-forming method (37) and a hemolysis method (37).

A very simple method is based on the foam-forming properties of saponins. A standard volume (e.g., 5 ml) of the saponin solution in 1/15 M dipotassium hydrogen phosphate is shaken for 1 min in a 25 ml measuring cylinder. The volume of foam remaining on the cylinder after it has stood for 1 min is then proportional to the concentration of saponin (37). This method has a major disadvantage in that it obviously relies on the complete absence of other surfactants, and it is not particularly sensitive. It can only be used to determine amounts of saponins in excess of about 500 μg (1).

Various quantitative methods using hemolysis have been reviewed by Birk (37). Hemolysis methods rely on the fact that a critical concentration of saponin (reported in grams of an isotonic salt solution per gram of saponins) is required to lyse erythrocytes. The maximum dilution of saponin is defined as the “hemolytic index.” Various amounts of the saponin-containing materials are mixed with a suspension of washed erythrocytes in isotonic buffer at pH 7.4. After 24 h the mixture is centrifuged and hemolysis is indicated by the presence of hemoglobin in the supernatant. The minimum amount of material that will produce hemolysis then gives the saponin concentration—provided that the hemolytic index for that particular saponin, or mixture of saponins, is known. The hemolytic index depends on both the nature of the saponin and the species of animal from which the erythrocytes were obtained, so it is essential to use standards prepared from a purified sample of the saponin, or mixture of saponins, that is being measured (1).

Hemolytic methods again have the disadvantage that they rely on the complete absence of other surface-active compounds that may also be hemolytic. Consequently, although very sensitive, they are unsuitable for routine testing of unknown plant materials (1).

The first method designed for determination of soybean saponins was described by Gestetner *et al.* (25). Defatted materials (either soybeans or soybean flour) are refluxed with 1 N H_2SO_4 in dioxane-water (1:3) for 4 h to hydrolyze the saponins. The sapogenins are extracted with three successive portions of ether purified on a column of Al_2O_3 . The concentration of sapogenin in a solution of the purified product can then be determined spectrophotometrically using a modified Liebermann-Burchard reagent (acetic acid/sulfuric acid; 3:2); a yellowish color develops immediately and changes to violet after a few seconds.

Thin-layer chromatography was also used to determine saponins. Quantitative results can be obtained in two ways. The density of the spots obtained with a suitable spray reagent can be measured directly using a densitometer (28,30). Saponin fractions prepared from the solvent extraction are spotted on a thin-layer chromatography plate, along with saponin standards. The plate, without solvent development, is directly treated with sulfuric acid and heat. The density of violet spots developed is proportional to the amount of saponins present (30). Alternatively, the

saponin spots can be determined by using iodine vapor, then scraped off into tubes and treated with concentrated sulfuric acid. The intensity of the brown color that is produced is then determined spectrophotometrically (106). The densities of the spots and the intensities of the colors produced by the test samples are then related to the densities and intensities produced from standard solutions of the saponin to provide a measure of the amount present in the unknown sample.

HPLC has been utilized as a tool for separation and quantification of saponins. A variety of detection methods have been used, such as UV, MS, Vis-NIR reflectance spectroscopy, and ELSD (Table 4.1). The triterpene glycosides were often hydrolyzed with subsequent analysis of the liberated sapogenins by HPLC using gradient elution and a mass detector (27). By use of a sapogenin/carbohydrate ratio, an estimate of the total saponin content was made. The mobile phase consisted of a light petroleum and ethanol. Both normal phase and reverse phase chromatography have been used. The mobile phases used in reverse phase were water and acetonitrile (90). In normal phase, chloroform containing 1% (v/v) acetic acid and methanol-water-acetic acid (95:4:1) were used (26).

After DDMP-conjugated soyasaponins B were discovered, UV detection was used due to their high absorbance at 292 nm. Examples of internal standards used when the UV detector was used are formononetin (29) and α -hederin (107). Most recently, the authors' laboratory has developed a method of using HPLC-ELSD to determine soyasaponins in their native forms (108). In this method, eight forms of soyasaponin B were quantitatively determined.

In summary, this chapter addressed structural characteristics, biological activities, and isolation and detection of saponins in soybeans. Saponins, a class of natural surfactants, are sterols or triterpene glycosides. They are present naturally in a wide variety of plants. Soy saponins are one of the most important sources of dietary saponins. Some biological activities of saponins were discussed. The mechanisms of different biological properties of the saponins that have been proposed were presented. Saponins are potentially functional food ingredients.

References

1. Oakenfull, D., Saponins in Food—A Review, *Food Chem.* 6:19–40 (1981).
2. Price, K.R., I.T. Johnson, and G.R. Fenwick, The Chemistry and Biological Significance of Saponins in Foods and Feedingstuffs, *CRC Crit. Rev. Sci. Nutr.* 26:27–135 (1987).
3. Shiraiwa, M., S. Kudo, M. Shimoyamada, K. Harada, and K. Okubo, Composition and Structure of 'Group A Saponin' in Soybean, *Agric. Biol. Chem.* 55:315–322 (1991a).
4. Shiraiwa, M., K. Harada, and K. Okubo, Composition and Content of Saponins in Soybean Seed according to Variety, Cultivation Year and Maturity, *Agric. Biol. Chem.* 55:323–331 (1991b).
5. Tsukamoto, C., A. Kikuchi, K. Harada, K. Kitamura, and K. Okubo, Genetic and Chemical Polymorphisms of Saponins in Soybean Seed, *Phytochemicals* 34:1351–1356 (1993).
6. Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, and K. Kitamura, Factors Affecting Isoflavone Content in Soybean Seeds: Changes in Isoflavones,

- Saponins, and Composition of Fatty Acids at Different Temperature during Seed Development, *J. Agric. Food Chem.* 43:1184–1192 (1995).
7. Liener, I.E., Implications of Antinutritional Components in Soybean Foods. *Crit. Rev. Food Sci. Nutr.* 34:31–67 (1994).
 8. Oakenfull, D., and G.S. Sidhu, Could Saponins Be a Useful Treatment for Hypercholesterolemia? *Eur. J. Clin. Nutr.* 47:79–88 (1990).
 9. Hendrich, S., T.T. Song, S.O. Lee, and P.A. Murphy, Are Saponins and/or Other Soybean Components Responsible for Hypocholesterolemic Effects of Soybean Foods? *J. Nutr.* 130:674S (2000).
 10. Matsura, H., Saponins in Garlic as Modifiers of the Risk of Cardiovascular Disease, *J. Nutr.* 131:1000S–1005S (2001).
 11. Konoshima, T., Anti-Tumor-Promoting Activities of Triterpenoid Glycosides: Cancer Chemoprevention by Saponins, *Adv. Exp. Med. Biol.* 404:87–100 (1996).
 12. Jeon, H.S., and M.K. Sung, Soybean Saponins Inhibit the Formation of DNA Adducts in Colon and Liver Cells, *J. Nutr.* 130:687S (2000).
 13. Oh, Y.J., and M.K. Sung, Soybean Saponins Inhibit Cell Proliferation by Suppressing PKC Activation and Induce Differentiation of HT-29 Human Colon Adenocarcinoma Cells, *Nutr. Cancer* 39:132–138 (2001).
 14. Nakashima, H., K. Okubo, Y. Honda, T. Tamura, S. Matsuda, and N. Yamamoto, Inhibitory Effect of Glycosides like Saponins from Soybean on the Infectivity of HIV in Vitro, *AIDS* 3:655–658 (1989).
 15. Okubo, K., S. Kudou, T. Uchida, Y. Yoshiki, M. Yoshikoshi, and M. Tonomura, Soybean Saponins and Isoflavonoids: Structure and Antiviral Activity against Human Immunodeficiency Virus in Vitro, *ACS Symp. Ser.* 546:330–339 (1994).
 16. Hayashi, K., H. Hayashi, N. Hiraoka, and Y. Ikeshiro, Inhibitory Activity of Soyasaponin II on Virus Replication in Vitro, *Planta Medica* 63:102–105 (1997).
 17. Rao, A.V., and M.K. Sung, Saponins as Anticarcinogens, *J. Nutr.* 125:717S–724S (1995).
 18. Sung, M.K., and M.Y. Park, Effect of Soybean Saponins on the Growth and Antioxidant Defense of Human Hepatocarcinoma Cells, *J. Nutr.* 130:687S (2000).
 19. Lasztity, R., M. Hidvegi, and A. Bata, Saponins in Food, *Food Rev. Int.* 14:371–390 (1998).
 20. Kitagawa, I., M. Yoshikawa, T. Hayashi, and T. Taniyama, Characterization of Saponin Constituents in Soybeans of Various Origins and Quantitative Analysis of Soyasaponins by Gas-Liquid Chromatography, *Yakagaku Zasshi [Journal of the Pharmaceutical Society of Japan]* 104:162–168 (1984a).
 21. Kitagawa, I., M. Yoshikawa, T. Hayashi, and T. Taniyama, Quantitative Determination of Saponins in Soybeans of Various Origins and Soybean Products by Means of HPLC, *Yakagaku Zasshi [Journal of the Pharmaceutical Society of Japan]* 104:275–279 (1984b).
 22. Kitagawa, I., M. Saitom, T. Hayashi, and T. Taniyama, Saponin and Sapogenol. XXXVIII. Structure of Soyasaponin A2, a Bisdesmoside of Soyasapogenol A, from Soybean, the Seeds of *Glycine max* Merrill, *Chem. Pharm. Bull.* 33:598–608 (1985).
 23. Yoshiki, Y., S. Kudou, and K. Okubo, Relationship between Chemical Structures and Biological Activities of Triterpenoid Saponins from Soybean, *Biosci. Biotechnol. Biochem.* 62:2291–2299 (1998).
 24. Kudou, S., M. Tonomura, C. Tsukamoto, T. Uchida, M. Yoshikoshi, and K. Okubo, *Structural Elucidation and Physiological Properties of Genuine Soybean Saponins, Food Phytochemicals for Cancer Prevention I: Fruits and Vegetables*, edited by Mou-Tuan,

- Huang, T. Osawa, Chi-Tang Ho, and R.T. Rosen, Oxford University Press, Oxford, U.K., 1994, pp. 340–348.
25. Gestetner, B., Y. Birk, A. Bondi, and Y. Tencer, Soya Bean Saponins—VII: A Method for the Determination of Sapogenin and Saponin Contents in Soya Beans, *Phytochemistry* 5:803–806 (1966).
 26. Ireland, P.A., and S.Z. Dziedzic, High-Performance Liquid Chromatography of Soya-saponins on Silica Phase with Evaporative Light-Scattering Detection, *J. Chromatogr.* 361:410–416 (1986a).
 27. Ireland, P.A., and S.Z. Dziedzic, Analysis of Soybean Sapogenins by High-Performance Liquid Chromatography, *J. Chromatogr.* 325:275–281 (1985).
 28. Fenwick, D.E., and D. Oakenfull, Saponin Content of Soya Beans and Some Commercial Soya Bean Products, *J. Sci. Food Agric.* 32:273–278 (1981).
 29. Hu, J., S. Lee, S. Hendrich, and P.A. Murphy, Quantification of the Group B Soyasaponins by High-Performance Liquid Chromatography, *J. Agric. Food Chem.* 50:2587–2594 (2002).
 30. Gurfinkel, D.M., and A.V. Rao, Determination of Saponins in Legumes by Direct Densitometry, *J. Agric. Food Chem.* 50:624–430 (2002).
 31. Rupasinghe, H.P.V., C.C. Jackson, V. Poysa, C.D. Berardo, J.D. Bewley, and J. Jenkinson, Soyasapogenol A and B Distribution in Soybean (*Glycine max* L. Merr.) in Relation to Seed Physiology, Genetic Variability, and Growing Location, *J. Agric. Food Chem.* 51:5888–5894 (2003).
 32. Daveby, Y.D., P. Aman, J.M. Betz, and S.M. Musser, Effect of Storage and Extraction on Ratio of Soyasaponin I to 2,3-Dihydro-2,5-dihydroxy-6-methyl-4-pyrone-conjugated Soyasaponin I in Dehulled Peas (*Pisum sativum* L.), *J. Sci. Food Agric.* 78:141–146 (1998).
 33. Ascherio, A., and W.C. Willett, New Directions in Dietary Studies of Coronary Heart Disease, *J. Nutr.* 125:647S–655S (1995).
 34. Malinow, M.R., P. McLaughlin, G.O. Kohler, and A.L. Livingstone, Prevention of Elevated Cholesterolemia in Monkeys by Alfalfa Saponins, *Steroids* 29:105–110 (1977).
 35. Oakenfull, D.G., D.E. Fenwick, R.L. Hood, D.L. Topping, R.J. Illman, and G.B. Storer, Effects of Saponins on Bile Acids and Plasma Lipids in the Rat, *Br. J. Nutr.* 42:209–216 (1979).
 36. Potter, J.D., R.J. Illman, G.D. Calvert, D.G. Oakenfull, and D.L. Topping, Soya Saponins, Plasma Lipids, Lipoproteins and Fecal Bile Acids: A Double Blind Cross-Over Study, *Nutr. Rep Int.* 22:521–528 (1980).
 37. Birk, Y., Saponins, in *Toxic Constituents of Plant Food Stuffs*, edited by I.E. Liener, Academic Press, New York, 1969, pp. 169–210.
 38. Lin, A., G. Krockmalnic, and S. Penman, Imaging Cytoskeleton–Mitochondrial Membrane Attachments by Embedment- Free Electron Microscopy of Saponin-Extracted Cells, *Proc. Natl. Acad. Sci.* 87:8565–8569 (1990).
 39. Akiyama, T., S. Takagi, U. Samkawa, S. Inari, and H. Saito, Saponin-Cholesterol Interaction in the Multibilayers of Egg Yolk Lecithin as studied by Deuterium Nuclear Magnetic Resonance: Digitonin and Its Analogues, *Biochemistry* 19:1904–1911 (1980).
 40. West, C.E., A.C. Beynen, K.E. Scholz, A.H.M. Terpstra, J.B. Schutte, K. Deuring, and L.G.M. Van Gils, Treatment of Dietary Casein with Formaldehyde Reduces Its Hypercholesterolemic Effect in Rabbits, *J. Nutr.* 114:17–25 (1984).

41. Oakenfull, D.G., D.L. Topping, R.J. Illman, and D.E. Fenwick, Prevention of Dietary Hypercholesterolaemia in the Rat by Soya Bean and Quillaja Saponins, *Nutr. Rep. Int.* 29:1039–1049 (1984).
42. Sidhu, G.S., and D.G. Oakenfull, A Mechanism for the Hypocholesterolemic activity of Saponins, *Br. J. Nutr.* 55:643–649 (1986).
43. Sugano, M., S. Goto, Y. Yamada, K. Yoshida, Y. Hashimoto, T. Matsuo, and M. Kimoto, Cholesterol-Lowering Activity of Various Undigested Fractions of Soybean Protein in Rats, *J. Nutr.* 12:977–985 (1990).
44. Shimoyamada, M., I. Shingo, R. Ootsubo, and K. Watanabe, Effects of Soybean Saponins on Chymotryptic Hydrolyses of Soybean Proteins, *J. Agric. Food Chem.* 46:4793–4797 (1998).
45. Shimoyamada, M., R. Ootsubo, T. Naruse, and K. Watanabe, Effects of Soybean Saponin on Protease Hydrolyses of β -Lactoglobulin and α -Lactalbumin, *Biosci. Biotechnol. Biochem.* 64:891–893 (2000).
46. Armstrong, B., and R. Doll, Environmental Factors and Cancer Incidence and Mortality in Different Countries with Special References to Dietary Practices, *Int. J. Cancer* 15:617–631 (1975).
47. Paxton, S.J., *Soybean and Consumption & Disease Incidence*, Preventive Nutrition Consultants, Inc., Seattle, Washington, 1998.
48. Reddy, B.S., Dietary Fiber and Colon Cancer, *Prev. Med.* 16:559–565 (1987).
49. Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences, National Research Council, *Diet and Health: Implications for Reducing Chronic Disease Risk*, National Academy Press, Washington, D.C., 1989.
50. Health and Welfare Canada, *Nutrition Recommendations: The Report of the Scientific Review Committee*, Author, Ottawa, 1990.
51. American Cancer Society, *Cancer Facts & Figures, 2004*. Available at www.cancer.org. Accessed August 6, 2004.
52. Organization for Economic Co-operation and Development, *Food Consumption Statistics*, DECD Publications, Paris, 1991.
53. Dunn, J.E., Jr., Cancer Epidemiology in Populations of the United States—with Emphasis on Hawaii and California—and Japan, *Cancer Res.* 35:3240–3245 (1975).
54. Ravikumar, P.R., H. Paul, and J.S. Charles, Cytotoxic Saponins from the Chinese Herbal Drug Yunnan Bai Yao, *J. Pharm. Sci.* 68:900–903 (1979).
55. Sati, O.P., G. Pant, T. Nohara, and A. Sato, Cytotoxic Saponins from Asparagus and Agave, *Pharmazie* 40:586 (1985).
56. Malinow, M.R., P. McLaughlin, C. Stafford, A.L. Livingstone, G.O. Kohler, and R.C. Peter, Comparative Effects of Alfalfa Saponins and Alfalfa Fiber on Cholesterol Absorption in Rats, *Am. J. Clin. Nutr.* 32:1810–1812 (1979).
57. Deschner, E.E., and A.P. Maskens, Significance of the Labeling Index and Labeling Distribution as Kinetic Parameters in Colorectal Mucosa of Cancer Patients and DMH Treated Animals, *Cancer* 50:1136–1141 (1982).
58. Maharaj, I., K.J. Froh, and J.B. Campbell, Immune Responses of Mice to Inactivated Rabies Vaccine Administered Orally: Potentiation by Quillaja Saponin, *Can. J. Microbiol.* 32:414–420 (1986).
59. Wu, C.Y., C.C. Hsu, S.T. Chen, and Y.C. Tsai, Soyasaponin I, a Potent and Specific Sialyltransferase Inhibitor, *Biochem. Biophys. Res. Commun.* 284:466–469 (2001).
60. Haridas, V., M. Higuchi, G.S. Jayatilake, D. Bailey, K. Mujoo, M.E. Blake, C.J. Arntzen, and J.U. Gutterman, Avicins: Triterpenoid Saponins from *Acacia victoriae* (Benth)

Induce Apoptosis by Mitochondrial Perturbation, *Proc. Natl. Acad. Sci.* 98:5821–5826 (2001).

61. Berhow, M.A., E.D. Wagner, S.F. Vaughn, and M.J. Plewa, Characterization and Antimutagenic Activity of Soybean Saponins, *Mutation Res.* 448:11–22 (2000).
62. Koratkar, R., and A.V. Rao, Effects of Soya Bean Saponins on Azoxymethane-Induced Preneoplastic Lesions in the Colon of Mice, *Nutr. Cancer* 27:206–209 (1997).
63. Wu, R.T., H.C. Chiang, W.A. Fu, K.Y. Chien, Y.M. Chung, and L.Y. Horng, Formosanin-C, and Immunomodulator with Antitumor Activity, *Int. J. Immunopharmacol.* 12:777–786 (1990).
64. Kenarova, B., H. Neycher, C. Hadjiivanova, and D. Petkov, Immunomodulating Activity Ginsenoside Rg₁ from *Panax ginseng*, *Jpn. Pharmacol.* 54:447–454 (1990).
65. Kinjo, J., K. Yokomizo, T. Hirakawa, Y. Shii, T. Nohara, and M. Uyeda, Anti-Herpes Virus Activity of Fabaceous Triterpenoidal Saponins, *Bio. Pharm. Bull.* 23:887–889 (2000).
66. Ishaaya, I., Y. Birk, A. Bondi, and Y. Tencer, Soybean Saponin IX—Studies of Their Effect on Birds, Mammals and Cold-blooded Organisms, *J. Sci. Food Agric.* 20:433–436 (1969).
67. Tsujino, Y., S. Tsurumi, Y. Yoshida, and E. Niki, Antioxidative Effects of Dihydro- γ -Pyranyl-Triterpenoid Saponin (Chromosaponin I), *Biosci. Biotechnol. Biochem.* 58:1731–1732 (1994).
68. Yoshikoshi, M., Y. Yoshiki, K. Okubo, J. Seto, and Y. Sasaki, Prevention of Hydrogen Peroxide Damage by Soybean Saponins to Mouse Fibroblasts, *Planta Medica* 62:252–255 (1996).
69. Bomford, R., Aluminium Salts: Perspectives in Their Use as Adjuvants, in *Immunological Adjuvants and Vaccines. NATO ASI Series A: Life Sciences Vol. 179, Proceedings of a NATO Advanced Study Institute on Immunological Adjuvants and Vaccines, June 24–July 5, 1988, Cape Sounion Beach, Greece*, edited by G. Gregoriadis, A. C. Allison, and G. Poste, Plenum Press, New York, 1989, pp. 35–41.
70. Freund, J., The Effect of Paraffin Oil and Mycobacteria on Antibody Formation and Sensitization. A Review, *Am. J. Clin. Pathol.* 21:645–656 (1951).
71. Hunter, R., M. Olsen, and S. Buynitzky, Adjuvant Activity of Non-ionic Block Copolymers. IV. Effect of Molecular Weight and Formation on Titer and Isotype of Antibody, *Vaccine* 9:250–256 (1991).
72. Lefrancier, P., M. Derrien, I. Lederman, F. Niff, J. Choay, and E. Lederer, Synthesis of Some New Analogs of the Immuno-adjuvant Glycopeptide MDP (N-acetyl-muramyl-L-alanyl-D-isoglutamine), *Int. J. Pep. Prot. Res.* 11:289–296 (1978).
73. Chinnah, A.D., M.A. Balg, I.R. Tizard, and M.C. Kemp, Antigen Dependent Adjuvant Activity of a Polydispersed β -(1,4)-linked Acetylated Mannan (Acemannan), *Vaccine* 10:551–557 (1992).
74. Kensil, C.R., S. Soltysik, D.A. Wheeler, and J.Y. Wu, Structure/Function Studies on QS-21, a Unique Immunological Adjuvant from *Quillaja saponaria*, in *Saponins Used in Traditional and Modern Medicine*, edited by G.R. Waller and K. Yamasaki, Plenum Press, New York, 1996, pp. 165–172).
75. Bomford, R., Saponin and Other Haemolysins (Vitamin A, Aliphatic Amines, Polyene Antibiotics) as Adjuvants for SRBC in the Mouse. Evidence for a Role for Cholesterol-Binding in Saponin Adjuvanticity, *Int. Arch. Allergy Appl. Immun.* 63:170–177 (1980).

76. Oda, K., H. Matsuda, T. Murakami, S. Katayama, T. Ohgitani, and M. Yoshikawa, Adjuvant and Haemolytic Activities of 47 Saponins Derived from Medicinal and Food Plants, *J. Biol. Chem.* 381:67–74 (2000).
77. Gestetner, B., Y. Birk, and Y. Tencer, Soybean Saponins: Fate of Ingested Soybean Saponins and the Physiological Aspect of Their Hemolytic Activity, *J. Agric. Food Chem.* 16:1031–1035 (1968).
78. Ohminami, H., Y. Kimura, H. Okuda, and S. Arichi, Effect of Soyasaponins on Liver Injury Induced by Highly Peroxidized Fat in Rats, *Planta Medica* 50:440–441 (1984).
79. Sasaki, K., N. Minowa, H. Kuzuhara, S. Nishiyama, and S. Omoto, Derivatization of Soyasapogenol A and Their Hepatoprotective Activities, *Bioorg. Med. Chem. Lett.* 8:607–612 (1998).
80. Kinjo, J., M. Imagire, M. Udayama, T. Arao, and T. Nohara, Structure-Hepatoprotective Relationship Study of Soyasaponins I–IV Having Soyasapogenol B as Aglycone, *Planta Medica* 64:233–236 (1998).
81. Yoshiyuki, K., and H. Okuda, Biochemical and Pharmacological Studies of Natural Products Isolated from Various Medicinal Plants and Foodstuffs, in *Studies in Natural Products Chemistry Vol. 27: Bioactive Natural Products (Part H)*, edited by Atta-ur-Rahman, Elsevier Science, Amsterdam, 2002, pp. 398–400.
82. Tencer, Y., S. Shany, B. Gestetner, Y. Birk, and A. Bondi, Titrimetric Method for Determination of Medicagenic Acid in Alfalfa (*Medicago sativa*), *J. Agric. Food Chem.* 20:1149–1151 (1972).
83. Jurzysta, M., and A. Jurzysta, Gas-Liquid Chromatography of Trimethylsilyl Ethers of Soya Sapogenols and Medicagenic Acid, *J. Chromatogr.* 148:517–520 (1978).
84. Ireland, P.A., S.Z. Dziedzic, and M. Kearsley, Saponin Content of Soya and Some Commercial Soya Products by Means of High-Performance Liquid Chromatography of Sapogenins, *J. Sci. Food Agric.* 37:694–698 (1986b).
85. Nowacka, J., and W. Oleszek, High-Performance Liquid Chromatography of Zanhic Acid Glycosides in Alfalfa (*Medicago sativa*), *Phytochem. Anal.* 3:227–230 (1992).
86. Oleszek, W., K.R. Price, I.J. Colquhoun, M. Jurzysta, M. Ploszynski, and G.R. Fenwick, Isolation and Identification of Alfalfa (*Medicago sativa* L.) Root Saponins: Their Activity in Relation to a Fungal Bioassay, *J. Agric. Food Chem.* 38:1810–1817 (1990a).
87. Oleszek, W., and M. Jurzysta, High-Performance Liquid Chromatography of Alfalfa Root Saponins, *J. Chromatogr.* 519:109–116 (1990b).
88. Oleszek, W., M. Junkuszew, and A. Stochmal, Determination and Toxicity of Saponins from *Amaranthus cruentus* Seeds, *J. Agric. Food Chem.* 47:3685–3687 (1999).
89. Nowacka, J., and W. Oleszek, Determination of Alfalfa (*Medicago sativa*) Saponins by High-Performance Liquid Chromatography, *J. Agric. Food Chem.* 42:727–730 (1994).
90. Crespin, F., M. Calmes, R. Elias, C. Maillard, and G. Balansard, High-Performance Liquid Chromatographic Determination of Saponins from *Hedera Helix* L. Using a Light-Scattering Detector, *Chromatographia* 38:183–186 (1994).
91. Fuzzati, N., B. Gabetta, B. Gabetta, K. Jayakar, R. Pace, G. Ramaschi, and F. Villa, Determination of Ginsenosides in *Panax Ginseng* Roots by Liquid Chromatography with Evaporative Light-Scattering Detection, *J. AOAC Int.* 83:820–829 (2000).
92. Ganzear, M., E. Bedir, and I.A. Khan, Determination of Steroidal Saponins in *Tribulus terrestris* by Reversed-Phase High-Performance Liquid Chromatography and Evaporative Light Scattering Detection, *J. Pharm. Sci.* 90:1752–1758 (2001).

93. Li, W., and J.F. Fitzloff, A Validated Method for Quantitative Determination of Saponins in Notoginseng (*Panax notoginseng*) Using High-Performance Liquid Chromatography with Evaporative Light-Scattering Detection, *J. Pharm. Pharmacol.* 53:1637–1643 (2001).
94. Massiot, G., C. Lavaud, L. Le Men-Olivier, G. Binst, S.F. Miller, and H.M. Fales, Structural Elucidation of Alfalfa Root Saponins by MS and NMR Analysis, *J. Chem. Soc. Perkins Trans.* 3071–3079 (1988).
95. Maillard, M.P., and K. Hostettmann, Determination of Saponins in Crude Plant Extracts by Liquid Chromatography-Thermospray Mass Spectrometry, *J. Chromatogr.* 647:137–146 (1993).
96. Fuzzati, N., R. Pace, G. Papeo, and F. Peterlongo, Identification of Soyasaponins by Liquid Chromatography-Thermospray Mass Spectrometry, *J. Chromatogr. A* 777:233–238 (1997).
97. Bialy, Z., M. Jurzysta, W. Oleszek, S. Piacente, and C. Pizza, Saponins in Alfalfa (*Medicago sativa* L.) Root and Their Structural Elucidation, *J. Agric. Food Chem.* 47:3185–3192 (1999).
98. Wang, X.M., T. Sakuma, E.A. Adjaye, and G.K. Shiu, Determination of Ginsenosides in Plant Extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS, *Anal. Chem.* 71:1579–1584 (1999).
99. Cui, M., F. Song, Y. Zhou, Z. Liu, and S. Liu, Rapid Identification of Saponins in Plant Extracts by Electrospray Ionization Multi-Stage Tandem Mass Spectrometry and Chromatography/Tandem Mass Spectrometry, *Rapid Commun. Mass Spectrom.* 14:1280–1286 (2000).
100. Ren, G.X., and F. Chen, Simultaneous Quantification of Ginsenosides in American Ginseng (*Panax quinquefolium*) Root Powder by Visible/Near-Infrared Reflectance Spectroscopy, *J. Agric. Food Chem.* 47:2771–2775 (1999).
101. Ruiz, R.G., K.R. Price, M.E. Rose, A.E. Arthur, D.S. Petterson, and G.R. Fenwick, The Effect of Cultivar and Environment on Saponin Content of Australian Sweet Lupin Seed, *J. Sci. Food Agric.* 69:347–351 (1995a).
102. Wolf, W.J., and B.W. Thomas, Thin Layer and Anion Exchange Chromatography of Soybean Saponins, *J. Am. Oil Chem. Soc.* 47:86–90 (1970).
103. Domon, B., A.C. Dorsaz, and K. Hostettmann, High-Performance Liquid Chromatography of Oleanane Saponins, *J. Chromatogr.* 315:441–446 (1984).
104. Burnouf-Radosovich, M., and N.E. Delfel, High-Performance Liquid Chromatography of Triterpene Saponins, *J. Chromatogr.* 368:433–438 (1986).
105. Birk, Y., A. Bondi, B. Gestetner, and I. Ishaaya, A Thermostable Hemolytic[**AQ5**] Factor in Soybeans, *Nature* 197(March):1089–1090 (1963).
106. Kartnig, T., R. Danhofer-Nöhammer, O. Wegschaidner, Spectrophotometric Analysis of Steroid and Triterpenoid Compounds, *Arch. Pharm.* 305:515–522 (1972).
107. Ruiz, R.G., K.R. Price, M.E. Rose, M.J.C. Rhodes, and G.R. Fenwick, Determination of Saponins in Lupin Seed (*Lupinus angustifolius*) Using High-Performance Liquid Chromatography: Comparison with a Gas Chromatographic Method, *J. Liquid Chromatogr.* 18:2843–2853 (1995b).
108. Lin, J., and C. Wang, Analytical Method for Soy Saponins by HPLC/ELSD, *J. Food Sci.*, in press.

Chapter 5

Soy Flour: Varieties, Processing, Properties, and Applications

KeShun Liu^a and William F. Limpert^b

^aUniversity of Missouri, Columbia, MO 65211, and ^bCargill, Inc., Minneapolis, MN 55440

Soybeans are versatile. Generally speaking, they can be used as food, feed, and industrial material. Two features distinguish food uses of soybeans in the East and in the West. In the Far East, for thousands of years, soybeans have been made into various types of food, including soymilk, tofu, and soy sauce. These foods, known as traditional soyfoods, are made from whole beans for direct consumption. They are still popular today, except that the traditional preparation has been modified by modern processing technology.

In the West, where the history of soybean production and utilization is only about 100 years old, soybeans have been used as food mainly in the form of oil and protein ingredients. Soy protein products are made primarily from defatted soy meal or flakes and come in four major types: flour, concentrates, isolates, and textured soy protein. Soy flour is made simply through milling defatted soy meal or dehulled whole beans. Since nothing is removed except for hulls and/or fat, its protein content is similar to the starting material, about 55% on a dry-matter basis (db). Soy protein concentrate is made by aqueous alcohol extraction or acid leaching of defatted soy flakes. The process removes soluble carbohydrates, and the resulting product has about 70% (db) protein. Soy protein isolate is produced by alkaline extraction followed by precipitation at an acid pH. It is the most refined soy protein product after removal of both soluble and insoluble carbohydrates. Therefore, it has a protein content of 90% (db). Textured soy proteins are made mainly by thermoplastic extrusion of soy flour or soy concentrate under moist heat and high pressure to impart a fibrous texture. The textured proteins come in many sizes, shapes, colors, and flavors, depending on the ingredients added and the processing parameters.

Soy protein products are not consumed directly as food. Instead, as versatile ingredients they are incorporated into virtually every type of food system, including bakery, dairy, meat, breakfast cereal, beverages, infant formula, and dairy and meat alternatives. In these food systems, soy ingredients not only boost protein content but also provide many functional properties. The common functionalities of soy protein products include solubility, water absorption and binding, viscosity control, gelation, cohesion, adhesion, elasticity, emulsification, fat absorption or repulsion, flavor binding, foaming, whipping, and color control.

Soy flour has the lowest cost among soy protein products because it is the least processed. Soy flour retains most nutrients from the original beans and is an excellent low-fat source of protein, isoflavones, other nutrients, and phytochemicals. Yet, like other soy protein ingredients, it offers many functional properties, and thus has wide applications. The discovery of health benefits of soy and the recent fervor for high-protein diets further drive applications of soy flour in various food systems. This chapter focuses on soy flour with respect to variety, processing, nutritional value, functional properties, and applications in various food systems, as well as current trends. Additional information can be found in the literature (1–11).

Varieties of Soy Flour and Processing Techniques

Soy flour comes in many types, resulting from different processing approaches and application requirements. Based on fat content, we have full-fat, low-fat, defatted, and refatted soy flour. Based on particle size, we have soy grits, soy flour, and very fine soy flour. Soy grits are coarse ground products and are further graded in terms of mesh size of U.S. standard sieves: coarse 10–20, medium 20–40, and fine 40–80. Soy flour is a fine ground product that can pass #100 mesh of U.S. standard sieves. Most defatted soy flour is ground to pass #200 mesh. Recently new varieties of soy flour that can pass a mesh size much finer than 200 have been marketed, some ranging from 400 to 1,000 mesh. Based on degree of heat treatment, we have enzyme-active soy flour and heat-treated (such as by roasting and steaming, etc.) soy flour. Soy flours are further divided into many types based on different protein solubility (commonly expressed as protein dispersibility index or PDI) resulting from various levels of heat treatment. Based on texture, we have regular soy flour and textured soy flour.

Defatted Soy Flour

Defatted soy flour is made from defatted soy flakes, which are a product of modern soy processing, commonly based on a solvent extraction process. [Figure 5.1](#) is a flowchart showing various steps of the process. Basically, soybeans are first cleaned, dried, cracked, and dehulled, then conditioned with steam and flaked by passing through flaking rollers. The flakes are conveyed to an extractor where oil is removed by countercurrent solvent extraction, with hexane as a common solvent (12,13).

Soybeans are first cleaned by passing through a magnetic separator to remove iron, steel, and other magnetically susceptible objects, followed by shaking on progressively smaller-meshed screens to remove soil residues, pods, stems, weed seeds, undersized beans, and other trash.

To remove the hull effectively, moisture content in the range of 10–11% is needed, which requires a drying process prior to dehulling. Heated air is distributed through the soybeans to achieve some loss of water, followed by cooler air, which removes the residual moisture-laden air. The moisture is typically allowed to equilibrate throughout the bean (tempering) for 1–5 days but for up to 20 days at some

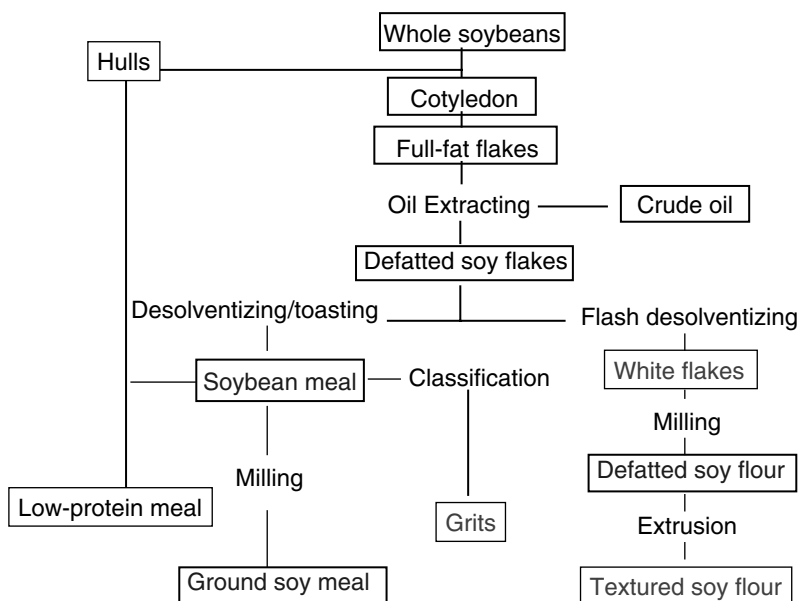


Figure 5.1. Flow chart for processing soybeans into defatted meal and flour.

plants. The beans may then be further screened and weighed before dehulling and preparation for oil extraction.

Cleaned and dried beans are cracked to break into small pieces for dehulling and flaking. Commercial cracking involves splitting open the soy hull between counter-rotating, corrugated, or fluted rollers. Cracking rollers are usually 25 cm in diameter and at least 107 cm long, processing up to 500–600 tons/day of soybeans. Cracking produces 4–6 cotyledon fragments, or “meats,” per bean. However, flour (fines) and larger fragments are also produced. The rollers are revolving at different speeds to produce a shearing action to tear the hull. The beans fall through a series of two or three rollers with the corrugations being fewer and smaller in the first roller and more frequent and larger in subsequent rollers.

The hulls are separated from the cotyledon fragments by aspiration. The meats are separated according to size on a vibrating screen and fines are removed by aspiration. Whole beans and larger fragments are sent back through the cracking mills. The hull stream is often sent through a secondary dehulling process to remove soy meats (cotyledons), typically including a secondary aspiration. However, fines are included with the meats for oil extraction to maximize extraction yield, even though they may create solvent filtration problems during oil extraction. Although soy cotyledons contain about 20% oil, soy hulls have negligible oil content. They are collected for use in animal feed.

Cracked soybeans (soy meats) must be conditioned by steam heating to obtain the optimum plasticity necessary for soy flake production, prior to oil extraction. The temperature of the hot flakes is 65–70°C. Steam heating raises the moisture content to 11%. The heaters commonly used are vertically stacked and rotary horizontal heat exchangers. Alternatively, fluidized bed heating dries the beans and conditions the meats with recirculated air providing rapid energy transfer and is more cost effective than conventional means. Controlling the bean and flake moisture minimizes the subsequent extraction of nonhydratable phospholipids by inactivating the enzyme phospholipase D (14).

The conditioned soy meats are flaked by passing between horizontal smooth rollers. The pressure is maintained by springs under hydraulic pressure producing flakes that are approximately 0.025–0.037 cm thick. The rollers are about 120 cm long and 70 cm in diameter. The rollers tend to wear more in the center than near the outer ends, which is a problem in preparing flakes of uniform thickness, unless care is taken in feeding the rollers evenly. The tensions on the springs are frequently adjusted and the rollers reground from time to time to maintain uniform flake production.

Flaking is the final, important step of bean preparation before solvent extraction. Solvent can flow much more readily through a bed of flakes, because of their higher surface area, than through a bed of soy meats. The passage between the rollers ruptures the oil-rich cotyledon cells, allowing improved solvent penetration to the lipid bodies. In addition, flaking reduces the diffusion distance solvent or miscella (oil/solvent) moves to extract oil.

Following flaking, oil is removed from the soy flakes by an organic solvent, commonly hexane, to form an oil/solvent mixture called a miscella. The oil is recovered from the miscella by removing the solvent by steam stripping. Solvent extraction of soybeans is a diffusion process in which the solvent (hexane) selectively dissolves miscible oil components. During extraction hexane rapidly solubilizes soy oil from cotyledon lipid bodies in soy flakes, as soon as it enters the lipid body. The slowest processes are solvent diffusion into the flake and diffusion of the oil/hexane miscella out of the flake. Nevertheless, this process is faster than extraction of raw cotyledons or fresh beans, which are almost impervious to solvent diffusion with hexane. Flake thickness is therefore very important in controlling diffusion, but flakes must be thick enough to avoid breaking up during handling. Crumbling of the thin flakes will result in fines, which will not allow the solvent to flow through as freely.

There are several types of solvent extractors available. Most commercial extraction is by continuous, countercurrent methods, using either deep-bed or shadow-bed extractors. In a typical deep-bed extractor system, soy flakes are added to rotating bins. The flakes are held in an upper chamber through which solvent percolates and drains out. At the end of the extraction process the flakes are dumped into a discharge chamber before addition of more flakes. Each bin is extracted by successively lower miscella concentration before a final hexane wash. A variation of

this system is a process whereby the flakes are stationary and the solvent sprays move to obtain a countercurrent system. Retention time depends on the rate of rotation and on the capacity of each cell rather than on the diameter of the extractor.

The defatted flakes remaining after extraction still contain about 30% residual solvent, which must be recovered. The system and conditions used for solvent removal, particularly with respect to time, temperature, and moisture, will determine the degree of protein denaturation in the flakes. One measure of the degree of protein denaturation is the protein dispersibility index (PDI). PDI essentially refers to the percentage of water-dispersible protein in a sample; the higher the PDI, the lower the degree of protein denaturation in the sample.

Because protein denaturation affects both nutritional value and functionality of finished products, different desolventizing systems are normally used for meal targeted mainly for animal feed and meal targeted for food use. In many processing plants, residual solvent is removed from the defatted flakes through a desolventizer-toaster (DT). This equipment removes the hexane by use of live steam. The steam that condenses furnishes the latent heat required for hexane evaporation, and the condensed stream raises the moisture level to a range of 16–24% to facilitate the toasting operation. The process is carried out at 100–105°C for 15–30 min. The flakes leaving the DT unit undergo drying and cooling steps. This can be accomplished in the same unit that includes the drying/cooking apparatus or in separate dryer/cooler equipment. The moisture of flakes is reduced to about 12% and the final temperature is less than 32°C. Because the flakes are subject to high-temperature moist heat during the toasting stage, protein denaturation takes place. The resulting meal has a low PDI value (PDI 15–25). With this PDI value, the meal has maximum nutritional value as animal feed but some functional properties are reduced or lost when used as a food ingredient. However, the product can be made into soy flour for food uses when high-quality beans and solvent are used and the system is kept clean during processing.

For minimizing soy protein denaturation, different desolventizing systems are required. The most commonly used system is a flash desolventizing system, in which superheated solvent gas is used to transport the solvent-saturated meal pneumatically and the transport gas is utilized to evaporate the solvent contained in the solid during a short contact time (2–5 s). The meal leaving this system via centrifugal separation is practically free of solvent except for the solvent contained in the pores (about 0.3 to 0.5%). At the same time, moisture in the flake is reduced by 3–5% while protein denaturation is minimized. Soy flakes processed in this way have PDI values as high as 90. The product is commonly known as white flakes.

In making food-grade soy meal, such as white flakes, extra attention should be paid to raw bean selection and preparation. The raw bean must be high quality, and any and all foreign materials must be removed through use of screening, aspiration, and other cleaning and sizing devices. In addition, the extractor must be specially designed with a self-cleaning feature. The right extraction temperature (about 60°C),

good percolation, and good hexane quality are the most important aspects for making good quality soy meal for food uses.

To remove the remaining solvent, a stripper is normally used in conjunction with a flash desolventizing system, using superheated steam under vacuum. The system is sometimes known as a vacuum desolventizing system. Through adjusting such processing parameters as pressure, live steam, moisture, and temperature, white flakes with a wide range of PDI (10–90) can be obtained. At or above atmospheric pressure, the steam condenses on the flakes, causing protein denaturation. Below atmospheric pressure (under vacuum), the steam does not condense and protein denaturation is avoided.

In the market, defatted soy flour is mostly available with PDI values of 20, 70, or 90. Soy flour with 20 PDI is the most heat processed, and has a toasted or nutty sensory note. Soy flour with 90 PDI has undergone the least heat treatment. Enzymes such as lipoxxygenase are not inactivated. It is also known as enzyme-active and thus will generate the most bitter and beany flavor upon hydration. Its use is primarily limited to bleaching wheat flour. Soy flour with 70 PDI is mildly heat-treated and compromises advantages and disadvantages between 20 and 90 PDI flour, and thus it becomes the most commonly used.

Heat treatment also inactivates trypsin inhibitors and some other biologically active compounds. Van den Hout *et al.* (15) studied inactivation kinetics of trypsin inhibitors in soy flour by measuring over a large range of temperatures (80–134°C) and moisture content (8–52%) and found that the inactivation of trypsin inhibitors showed a two-phase kinetic behavior. The influence of moisture content on the inactivation rate was larger at moisture content less than 30%.

Removal of lipid fractions during production of defatted soy flour leads to concentration of the other components. The protein content increases to over 50% and total carbohydrate content rises to over 30%. However, there is variability in soy flour composition due to changes in soybean variety and processors (16). After solvent removal, the defatted flakes are passed through grinders to produce coarse particle size for grits or milled to produce fine particles for defatted soy flour (17).

Refatted or Relecithinated Soy Flour

Refatted or relecithinated soy flour is made by blending fluid lecithin and refined soybean oil with defatted soy flour, resulting in a soy flour product with a total fat content of 3–15%. It has much improved properties of emulsification and dispersion.

Full-Fat Soy Grits and Soy Flour

Full-fat soy grits and flour are produced by grinding or milling dehulled soybeans. Thus their composition is identical to soybean cotyledon tissue, with protein at about 40% and fat at about 20%.

The starting material for the production of full-fat soy flour is a high-quality soybean. The beans are first cleaned and foreign seeds are removed by a combina-

tion of brushing, air aspiration, and screening. For production of enzyme-active full-fat soy flour, the beans are crackled through rollers, and the seedcoats are removed by dehulling and air aspiration. The cotyledon tissue is then milled to produce full-fat soy flour with different particle sizes.

Milling of the cotyledon to produce full-fat grits and flour is generally accomplished in a series of grinding stages that may or may not include sifting in between (17). The coarser fractions (grits) are generally produced by grinding through a roller mill. To produce finer flour, grits may be milled through a variety of fine-grinding machines. Due to the high oil content and relatively plastic nature of the full-fat soy flour, roller mills are not normally used. Hammer mills, pin mills, and a variety of air-swept pulverizers may be used. Because of the high energy input and sticky nature of the flour, the process equipment needs to be oversized to ensure the operating mechanism. Full-fat products are difficult to pulverize or to screen. It is customary to do the grinding in two steps and to separate the coarse from the fine particles in an air classifier between grindings. In this case, fine flour with particle size passing 100 or 200 mesh is collected for packing while coarse particles are returned to the grinder.

Full-fat soy flour is known as enzyme-active when heat treatment is kept minimal during all the stages of processing. Soy protein is highly soluble and functional in this type of product. Yet, the product has strong beany flavor when exposed to water due to action of naturally present lipoxygenase in soybeans.

In order to minimize development of beany flavor by lipoxygenase and improve nutritional value by eliminating certain naturally occurring antinutritional compounds in soybeans, whole soybeans are heat treated before milling. The resulting product is heat-treated full-fat soy grits or flour. A common heat treatment is roasting. Another common type of heat treatment is steaming. Cleaned soybeans are subject to a continuous water-washing step. This step preconditions the beans by causing a small increase in the moisture content. The beans then pass through continuous pressure cookers. The cooked beans are then dried, cooled, and dehulled before milling. Extrusion cooking (18) and ultrasound (19) have been reported for making heat-treated full-fat soy flour. Most heat treatments, although they improve flavor profile and nutritional values, cause protein denaturation to such a degree that the final product has a PDI of around 20. Ferrier and Lopez (20) reported an alternative method to prepare full-fat flour. It involves conditioning soybeans to about 23% moisture by soaking for 10 min and tempering for 1 h, heating in an air drier at 99°C for 25 min or 110°C for 15 min, and grinding to a powder. The resulting flour was claimed to have a bland flavor yet with a PDI between 40 and 55.

Low-Fat Soy Flour

Low-fat soy flour is made by dry extrusion of whole or dehulled soybeans at field moisture content, followed immediately by passing through a horizontal press to separate oil from meal. The expressed oil is a fine and premium product.

It has a low phospholipid content ($<0.2\%$), and can be consumed without further processing. If yellowish color is objectionable in some markets, the oil can be refined.

The resulting meal has a residual oil content of about 5–7%, and can be milled into low-fat soy flour. During the extrusion, the temperature reaches as high as 150°C , and the protein is well denatured; thus the meal has a very low PDI value (<20). Yet, the flour has a protein content of 50% on a dry-matter basis, and can be used as a food ingredient for various applications, primarily for bakery products. It can also be used as a raw material for textured soy protein as well as an ingredient for co-processing with cereal grains into snacks.

Compared with defatted 90 PDI soy flour and enzyme-active full-fat soy flour, low-fat soy flour has superior flavor since during the extrusion processing, enzymes responsible for bitter and beany flavor formation are effectively inactivated. Furthermore, the extrusion cooking parameters can be adjusted so as to impart a pleasant nutty flavor to the meal and result in meals with a wide range of PDI (14–60).

The origin of this work dates back to 1987 when Nelson *et al.* (21) at the University of Illinois were using dry extruders to press full-fat soybeans for human consumption. When whole or dehulled soybeans at field moisture content were cracked and extruded, the extrudate discharged in semi-fluid consistency. The material reverted to a dry and mealy consistency soon after exiting the extruder. Microscopic examination of the extrudate showed that the extrusion process disrupted the cell structure of the soybean cotyledon. Consequently, the oil was released from the naturally protected environment within the oil body into the matrix. It was proposed that the short time window before the oil gets reabsorbed into the matrix offers opportunity to press out the oil by mechanical means. Bench level and pilot plant level studies were followed to determine the feasibility of extracting oil by hydraulic pressing and screw pressing immediately after extrusion. It was demonstrated that approximately 70% oil recovery was feasible in a single pass through a screw press when the soybean extrudate was pressed immediately after extrusion. However, the extraction rate fell drastically when the extrudate was allowed to cool before pressing (9).

Later on, the technology was further developed and marketed by a commercial company, Insta-Pro International (Des Moines, IA). The system is not a simple screw press since the latter is generally applied to high-oil-bearing seeds, not soybeans. Instead, it is a combination of a dry extruder and a horizontal press. Since its development in the late 1980s, it has served as an alternative low-cost processing technology for solvent extraction of many oilseeds, including soybeans, cotton seeds, sunflower, and rapeseed. It is particularly suitable for rural areas in developing countries where oilseed production volume is small and capital resources are limited for building a solvent extraction plant. Chapter 10 covers details of this technology as well as of the resulting oil and low-fat soy meal.

Textured Soy Flour

Defatted soy flour can be further processed into a variety of structured forms through an extrusion process known as thermoplastic extrusion. The process imparts a fibrous texture, improved eating quality, and visual appeal in food products. Defatted soy flour is mixed with water, color, and flavors, and then it is fed at a controlled rate into an extruder-cooker. Extruders of both single-screw and twin-screw configuration are used. In the extruder barrel, the mixture is subjected to increasing temperature and pressure as mechanical work is applied. This causes the formation of films of denatured protein, which bind together. The mass then extrudes through restriction dies at the end of the extruder barrel. The sudden reduction in pressure causes expansion of the product. The expanded mass is immediately cut to size, dried, cooled, and packaged. Through this process, a wide range of products of varying size, shape, color, texture, and flavor can be obtained. Because the starting material is defatted soy flour, the composition of textured soy flour is close to that of defatted soy flour (22,23).

Textured soy flour is often called TSP (textured soy protein). Rehydration of the product yields a product that has a chewy meat-like texture that is useful as a meat extender and meat replacer. Textured soy has a great crunchy texture useful in bars and cereals (24).

Functional Properties, Nutritional Value, and Health Benefits of Soy Flour

Over the years, various types of soy flour have found application in various food systems. By incorporating into food systems, soy flour contributes certain functionality, nutritional value, and health benefits (1,2,5,25). In addition, the low cost of soy flour makes it a top choice among soy protein products for some applications (11).

Functional Properties

Proteins, by virtue of diverse physicochemical properties resulting from the nature and flexibility of their structure, provide various functional attributes in a food system. The noncovalent forces (electrostatic, hydrogen bonding, and hydrophobic interactions) of amino acid sidechains, together with covalent disulfide links between thiol groups of cysteine residues, are responsible for protein conformations. The chemical and biological functions of a protein depend solely on these interactions, the secondary and tertiary structure, and the exposed surface groups of amino acid sidechains. Functional properties of proteins can be defined as the physicochemical properties and their behavior in a food system, including interactions with other food components. The common functionalities of soy protein products include solubility, water absorption and binding, viscosity control, gelation, cohesion/adhesion, elasticity, emulsification/stabilization, fat absorption, flavor binding, foaming, whipping, and color control. These functionalities are attributed to the soy protein's

polymer chains, which contain lipophilic, polar, and nonpolar, as well as negatively and positively charged, groups, which enable soy protein to associate with many different types of compounds (26,27).

Solubility is one of the most basic physical properties of proteins, and a prime requirement for any functional application. Most often, a highly soluble protein is desirable for optimum functionality. Solubility of a protein under specified conditions is governed by the factors that influence the equilibrium between protein-protein and protein-water interactions. The most important factor affecting protein solubility is heat treatment. For example, the moist heat treatment, which is necessary to inactivate lipoxygenase and trypsin inhibitors in soy products, leads to insolubility of soy protein. Soy protein products with a range of solubility are available for different food uses.

Proteins, due to their amphiphilic character, possess emulsifying properties. An emulsion is a dispersion of oil droplets in a continuous aqueous matrix. Solubility and hydrophobicity of proteins play major roles in determining emulsifying properties. The ability of soy protein to aid formation and stabilization of emulsions is essential for many food applications, including coffee whiteners, mayonnaise, salad dressings, frozen desserts, and comminuted meats.

Gelation refers to the ability of proteins to form gels. Protein gels consist of a three-dimensional network in which water is entrapped. The basic factors that affect soy protein gelation include protein concentration; temperature, rate, and duration of heating; and cooling conditions. Soy flour and concentrates form soft fragile gels, while soy isolates form firm, hard, resilient gels. Protein gels form the basis for comminuted sausages and oriental textured food products. The ability of gel structure to provide a matrix to hold water, fat, flavor, sugar, and other food additives is very useful in a variety of food products.

Water binding capacity refers to the amount of water bound by protein. The bound water includes all hydration water and some water loosely associated with protein molecules following centrifugation. The amount of bound water generally ranges from 30 to 50 g/100 g protein. Factors that affect water binding of proteins include amino acid composition, protein structure and conformation, surface charge and polarity, ionic strength, pH, and temperature. Soy isolate has the highest water binding capacity (about 35 g/100 g) among soy protein products, due to its high protein content. Water holding capacity (WHC) is a measure of entrapped water, which includes both bound and hydrodynamic water. In general the WHC of soy flour and soy concentrate varies from 2 to 5 parts water to 1 part protein, depending on the processing method utilized. Soy protein isolate can have a WHC as high as 5 to 7 parts water to 1 part protein. Water holding capacity of proteins is very important in meat analogs, since it affects the texture, juiciness, and taste.

Nutritional Value and Health Benefits

Nutritional value of soy flour products is their ability to supply good-quality protein, oil, and carbohydrates as well as minerals and vitamins. The health benefits of soy flour refer to its ability to promote health and prevent diseases. Soy proteins,

isoflavones, and other phytochemicals are key components responsible for the documented health benefits of soy (28). Chapter 1 discusses the chemical composition, nutritional value, and health benefits of soybeans. Soy flour is the least-refined soy product. In producing various types of soy flour, only the hulls and/or part or total lipids are removed. Therefore, most nutrients in the original beans end up in soy flour products. Table 5.1 shows the approximate composition of soy flour along with that of other types of soy protein products. [Figure 5.2](#) shows isoflavone content in selected soy products. Soy flour has the highest levels of nutrients compared with soy concentrates and isolates, except for protein content.

Low Cost

Since soy flour is the least processed among soy protein products, it is also the most economical. Soy grit and flour products have the lowest cost among soy protein ingredients in terms of price per unit of protein content ([Fig. 5.3](#)). Furthermore, when used as a replacer for eggs, milk, and other animal proteins, cost reduction is obvious since soy flour is less expensive than animal proteins.

Effects of Processing

It should be emphasized that processing alternatives enable us to have soy products with varying degrees of heat treatment and granulations. These variables significantly affect the functional properties of the final flour products as well as their nutritional value. Fully toasted products have optimal nutritional value; untoasted products have maximal functionality. By closely controlling the heat treatment and

TABLE 5.1
Typical Composition of Various Soy Protein Products^a

	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Crude fiber (%)	Ash (%)
Defatted soy flour	6.0	52.5	2.8	32.2	2.5	6.5
Textured soy flour	6.0	52.5	2.8	32.2	2.5	6.5
Full-fat soy flour	6.0	38.0	22.0	28.0	3.0	6.0
6% Releicithinated soy flour	6.0	47.0	7.0	34.0	2.3	6.0
Soy protein concentrate	5.5	67.3	2.7	19.5	4.0	5.0
Soy protein isolate	4.6	88.5	2.6	0.0	0.1	4.2

^aData from Godfrey, 2002 (28). All measurements were on an as-is basis. Fat was measured by acid hydrolysis. Carbohydrate was determined by difference calculation.

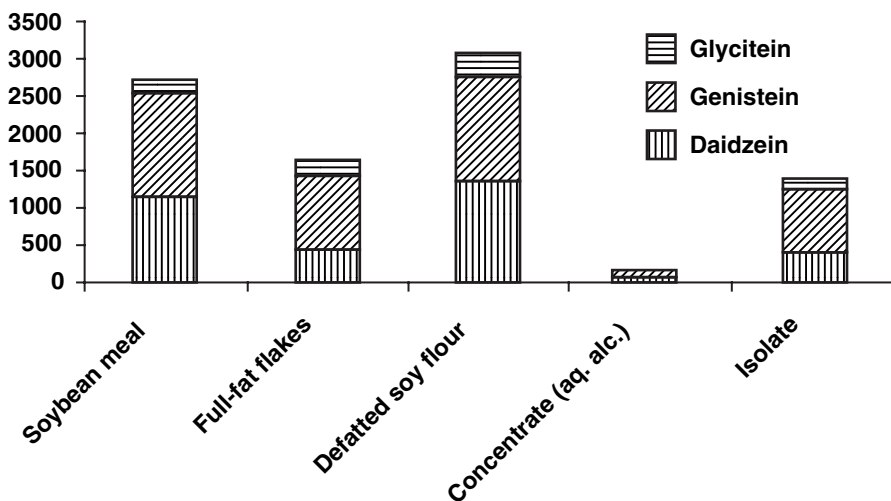


Figure 5.2. Isoflavones in selected soy products.

mechanical treatment (grinding or shearing during extrusion), it is possible to regulate the functional and nutritional properties of soy flour so that they are optimized for each application. This also explains why different types of soy flour have different applications in food systems.

Food Applications of Soy Flour

Soy flour is a nutritious, functional, and economical food ingredient. Soy flour may be used to enhance nutrition, to replace traditional ingredients, or to lower produc-

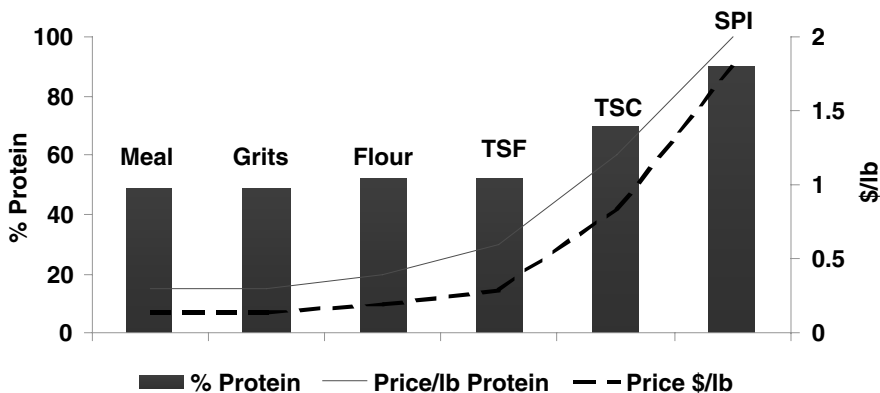


Figure 5.3. Prices of soy ingredients vs. protein content. Meal, grits, and flour are all defatted. TSF, textured soy flour; TSC, textured soy concentrate; SPI, soy protein isolate.

tion costs, and has thus found wide application in a wide variety of food products (2,11). Bakers have long understood soy flour to provide moisture retention, whiten crumb color, darken crusts, extend shelf life, shorten baking time, and decrease fat absorption. Soy flour can also be an excellent low-fat source of isoflavones as well as of protein, by providing all the amino acids essential for human health. Soy flour is also utilized in blends with inexpensive dairy components or to replace nonfat dry milk or other costly dairy components in many formulations. Many egg components can be replaced by soy proteins, such as the use of lecithinated soy flour to substitute for a substantial percentage of egg yolk solids. Naturally low-protein pasta products, such as spaghetti, can be fortified with soy flour to increase their nutritional value. Breakfast cereals and bars now use soy proteins (powder or texturized) to boost protein quality and quantity. Many of these uses are further enhanced in several nations by the ability to utilize a soy health claim (30). It allows for the addition of specific levels of soy proteins to foods for the claim of potential reduction of coronary heart disease. A number of common bakery and cereal products, including bread, can now be formulated to contain substantial levels (about 35% by weight) of various soy protein products.

Table 5.2 lists typical applications of various commercial soy flour products, along with inclusion levels and impact on certain functionality. The key selection factors are functionality, nutritional value/health claims, flavor profile, availability, price, fat content, particle size, and structural properties. In some cases, different flours can be used interchangeably. In other cases, a specific application requires a specific product.

Full-Fat Soy Flour

Enzyme-active full-fat soy flour is used mainly in the baking industry. In many European countries, over 90% of bread is produced with the use of enzyme-active soy flour, normally at a concentration less than 1%. The key functional component is lipoxxygenase. The enzyme can bleach wheat flour and condition dough through catalyzing oxidative reactions, leading to considerable improvement in crumb color, texture, and keeping quality of white bread. Another active enzyme in the soy flour is beta-amylase. Soy beta-amylase is more heat stable than that of wheat or barley and remains active longer in the early stages of baking, also contributing to improved texture (3).

In some cases, full-fat grits may be conditioned and flaked to improve hydration and reduce soaking time in the production of soymilk and tofu products (31). Flours may also be used in these kinds of applications. Grits and flakes may be further conditioned and cooked in an extruder to produce textured soy proteins. The latter can be used as meat analogs and extenders. However, defatted flour and grits are more commonly used for texturization.

Heat-treated full-fat soy flour is used as both functional and nutritional ingredients in a wide variety of food products. Full-fat soy flour is used extensively in many bakery products, such as cake, bread, pastry, and biscuits as a partial replacer for

TABLE 5.2

Typical Commercial Uses of Soy Flour Products (11)

Product	Typical applications	Functionality	Typical inclusion
Grits (50% protein)	Fermentation feedstock for soy sauce, food enzymes, meats	Nitrogen source for fermentation, meat extender	Varies
Defatted flakes (50% protein)	Raw material for concentrate/isolate production		
Full-fat flour (35–37% protein)	Bakery applications, especially in Europe	Protein enhancer, egg/milk replacer	1–5% of dry ingredients
Defatted flour, 90 PDI (50% protein)	Bakery applications	Crumb whitener, dough conditioner	<0.5%
Defatted flour, 70 PDI (50% protein)	Waffle/pancake mixes, breads, doughnuts, tortillas, bagels	Water absorption and retention, fat repulsion, protein enhancement, improved cell structure/crumb	1–5% of dry ingredients
Defatted flour, 20 PDI (50% protein)	Various bakery applications, milk replacer	Water absorption and retention, replacement of milk/egg proteins, nutty flavor	1–5% of dry ingredients

eggs, milk, and other ingredients. The flour increases water absorption, stabilizes the structure, makes the crumb of cakes soft and moist, and greatly extends shelf life. In soups, gravies, and sauces, the inclusion contributes extra fat in a finely dispersed form that remains stable during processing. The nutritional attributes of full-fat soy flour have led to its considerable usage in baby foods, health foods, and fortified breakfast cereals.

With their gritty character, full-fat soy grits are highly suitable for production of mixed-grain bread in which milk acidification is required. It was the use of full-fat soy grits that first made it possible to produce breads with admixtures of up to 30% soy flour without any disadvantages from the point of view of taste or baking problems. Soy breads are becoming increasingly popular in the West. It is not only a welcome addition, but also a healthy one.

With a full-fat soy product capable of being modified in many ways, new possibilities are opened up in the field of product development. It is now possible to make products such as ice cream, instant drinks, and cheese-like products, in which the sole protein source is full-fat soy protein. The present state of soy flour processing opens up many new possibilities to the food industry.

Defatted Soy Flour

Defatted soy flour and grits, with their varying range of PDI values, are used in a great variety of applications in the food industry. High-PDI flour, which contains the soybean enzymes in an active form, is used extensively in the U.S. baking industry in the same way as enzyme-active full-fat soy flour is used in Europe. High-PDI defatted soy flour and flakes are also used as starting materials for the manufacture of other soy protein products, such as soy concentrate and soy isolate.

Defatted soy flours with low and medium PDI are mainly used in the baking industry. In breads, buns, rolls, cakes, and pancakes, soy flour improves moisture retention. In doughnut manufacture, soy flour can lead to a reduction of fat absorption during the frying process. Some of these functional properties change with PDI. A study carried out at a private U.S. company (Cargill) indicated that fat absorption in doughnuts containing 3% soy flour decreases as the PDI value of the soy flour increases (Fig. 5.4). There is also a relationship between water absorption of batter and PDI value of soy flour when soy flour is utilized at 3% in the formula (Fig. 5.5). Because of the increase in water absorption, soy flour inclusion increases batter yield when used at levels above 1% (Fig. 5.6). This effect is valuable to frozen pancake manufacturers who sell complete products.

In cookies, cakes, pancakes, doughnuts, and other pastry products, defatted soy flour is used as an alternative to egg or milk solids, with equal functionality. In another Cargill study, defatted soy flour can replace up to 25% of the eggs in a rich premium muffin formula (Fig. 5.7) and up to 50% in a lean recipe. In pasta, soy flour improves the machinability of dough. This is because dough containing soy flour is less sticky than dough made with 100% semolina, and the absorptive properties of

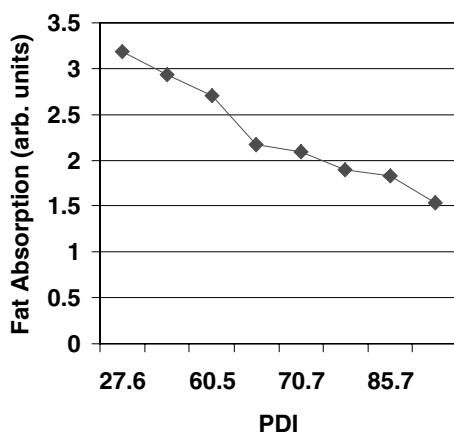


Figure 5.4. Fat absorption in doughnuts vs. protein dispersibility index (PDI) of incorporated soy flour (3% level).

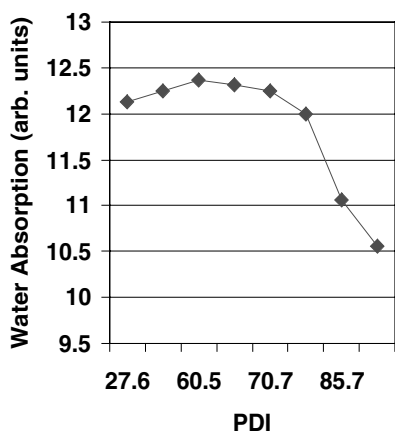


Figure 5.5. Water absorption in doughnuts vs. protein dispersibility index (PDI) of incorporated soy flour (3% level).

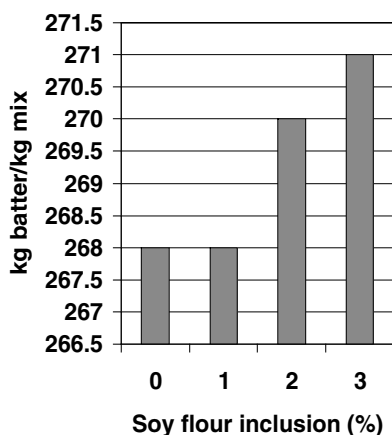


Figure 5.6. Batter yield vs. soy flour inclusion level.

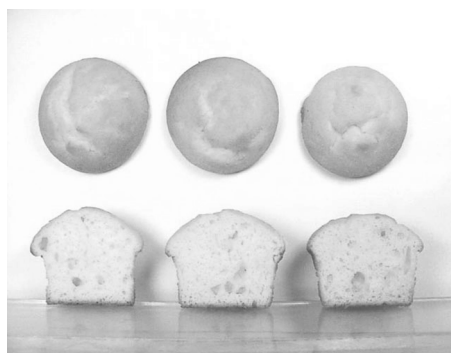


Figure 5.7. Muffins made with defatted soy flour to replace eggs. Courtesy of Cargill, Inc.

soy flour facilitate the rolling and cutting of pasta dough. Based on an in-house study at Cargill, pasta with 15–24% soy flour inclusion looks like standard pasta and flavor is basically unchanged. Toasted (low-PDI) defatted flour adds color to the crumb and a nutty toasted flavor to whole-grain and specialty breads. Up to 15% of toasted defatted flour can be added to quick-leavened bread.

Defatted soy flour and grits are also widely used in ground and comminuted meat products. In these systems, soy flour binds excess fat and water, cooking losses are reduced, and the size and shape of the meat products are better main-

tained on cooking. The coarse particles of grits also impart some texture to the finished products.

Textured Soy Flour

Similar to textured soy concentrate, textured soy flour is used mainly as an extender in meat products as well as pet foods. It contributes visual appeal to meat products and its unique structure gives a mouthfeel similar to diced or ground meat, thus complementing the eating quality of the meat products. Like regular soy flour, the textured products absorb water and fat and help reduce cooking loss, which results in the prevention of shrinkage during processing. The products also provide organoleptic appeal in many other foods such as snack foods and confectionery bars. It is also a major ingredient in meat analogs, providing high-quality protein as well as imparting a meat-like mouthfeel. Breaded chicken patties with as much as 30% of the meat replaced with hydrated textured soy flour were actually preferred to all-meat patties by a majority of participants in a consumer sensory test conducted at the Indiana State Fair (23).

Textured soy flour can also be consumed directly as simulated meat analogs, after using proper processing parameters, flavoring, and forming into various shapes, such as sheets, disks, patties, strips, and other shapes. In the market, there are meat-free meat analogs, such as hot dogs, hamburgers, chicken patties, hams, and sausages that are difficult to distinguish from the real ones.

Low-Fat Soy Flour

Low-fat soy flour has a protein content of 48–50% on a dry-matter basis, and can be used as a food ingredient for various applications, mostly for bakery products, including bread (up to 15%), cookies (24%), cakes (up to 25%), and muffins (up to 20%). It can also be used as a raw material for textured soy protein as well as an ingredient for coprocessing with cereal grains into snacks. Some of its applications parallel those of defatted or full-fat soy flour. Additional information can be found in Wijeratne (9) and Chapter 10 of this book.

Current Trends in Using Soy Flour

The use of soy flour in various food systems is not new. Yet, in the United States, the FDA-approved soy health claim of 1999 (30) opened up a new wave of the incorporation of soy protein products, including soy flour, into food systems. More recently, a rise in the popularity of low-carbohydrate diets, regardless of the validity of its scientific basis and sustainability in the marketplace, has caused a huge rush to incorporate soy protein in products in the United States. As a result, there have been several fundamental changes or emerging trends in using soy protein products in general and soy flour in particular in recent years. First, there is a shift of rationales for incorporating soy. In the past, the main objective for incorporating soy protein ingredients was to impart certain functional properties to a food system. Enrichment

of protein and other nutrients came as a secondary purpose, particularly in affluent societies where protein malnutrition is not a problem. Yet, due to medical discoveries about health benefits of soy and a new rush to increase protein content in foods in the midst of low-carbohydrate diet fever, the main rationale for incorporating soy protein ingredients has shifted to enrichment of foods with soy protein, isoflavones, and other phytochemicals. The improvement in food functionality is considered a secondary objective in many cases of current soy applications.

The second trend has been an increase in levels of incorporation. In the past, soy flour incorporation was at low levels (1–5%) since, with such levels, certain functional properties could be achieved. At this time, with the rationales changed to protein enrichment and health promotion, a much higher level of soy is needed in order to meet a certain level of soy protein in a food system.

Yet, high levels of incorporation have presented enormous challenges for food technologists, who are constantly struggling to maintain a balance between protein content, functional properties, beany taste, and other organoleptic properties. This leads to a third trend, that is, an increase in the use of different combinations of soy protein products. This serves as an effective way to meet the challenges of balancing different quality parameters in the final food products.

A fourth trend has been a wider application of soy protein products. Virtually every food item has now been tried with some type of soy protein incorporation. Thousands of new products, containing varying levels of soy protein, are being put into the market. Market positioning and profit enhancement are some of the key reasons for all these trends, since foods with high protein, particularly high soy protein, are now marketed in most cases at higher prices.

Conclusions

Soy flour is the least-processed soy protein ingredient product, and comes in many forms. It has a wide range of food applications due to its functionality, nutritional value, health benefits, and low cost as compared to soy protein concentrate and isolate. Market trends require food technologists to learn how to incorporate soy flour at high enough levels to induce health benefits without adversely affecting taste. They also must learn how to work with various forms of soy flour to gain maximum performance, and how to incorporate soy flour in a variety of food products. We hope that this chapter provides some clues for meeting these challenges. However, in the future, further developments in processing and application technology as well as new innovations will be needed.

References

1. Fulmer, R.W., The Preparation and Properties of Defatted Soy Flours and Their Products, in *Proceedings of the World Congress: Vegetable Proteins Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 55–61.

2. Fulmer, R.W. Uses of Soy Proteins in Bakery and Cereal Products, in *Proceedings of the World Congress: Vegetable Proteins Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, American Oil Chemists' Society, Champaign, Illinois, 1989, pp. 424–429.
3. Heiser, J., and T. Trentelman, Full-Fat Soya Products—Manufacturing and Uses in Foodstuffs, in *Proceedings of the World Congress: Vegetable Proteins Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, American Oil Chemists' Society, Champaign, Illinois, pp. 52–54.
4. Kanzamar, G.J., S.J. Predin, D.A. Oreg, and Z.M. Csehak, Processing of Soy Flours/Grits and Textured Soy Flour, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, edited by T.H. Applewhite, AOCS Press, Champaign, Illinois, 1993, pp. 226–240.
5. Lusas, E.W., and K.C. Rhee, Soy Protein Processing and Utilization, in *Practical Handbook of Soybean Processing and Utilization*, edited by D.R. Erickson, AOCS Press, Champaign, Illinois, 1995, pp. 117–160.
6. Hettiarachchy, N., and U. Kalapathy, Soybean Protein Products, in *Soybeans: Chemistry, Technology, and Utilization*, edited by K. Liu, Aspen Publishers, Gaithersburg, Maryland, 1999, pp. 379–411.
7. Endres, J., *Soy Protein Products, Characteristics, Nutritional Aspects and Utilization*, AOCS Press and Soy Protein Council, Champaign, Illinois, 2001.
8. Liu, K., Soy Flour: Variety, Processing and Applications, in *Proceedings, the Soy Protein Utilization Conference (June 17–19, Shanghai, China)*, American Soybean Association, St. Louis, Missouri, 2001, pp. 22–41.
9. Wijeratne, W.B., Non-solvent Technology in Soybean Processing, in *Proceedings of VII World Soybean Research Conference and IV International Soybean Processing and Utilization Conference*, Foz do Iguassu, Brazil, February 29–March 5, 2004, pp. 1146–1151.
10. Limpert, W.F., Soy Use in Energy Bars, Cereals, Snack Food and Bakery Goods, presented at Soyfoods Summit 2003, Miami, Florida, February 26–28, 2003.
11. Limpert, W.F., Soy Ingredients in Bakery and Other Cereal Products, presented at IV International Soybean Processing and Utilization Conference, Foz do Iguassu, Brazil, February 29–March 5, 2004.
12. Erickson, D.R., *Practical Handbook of Soybean Processing and Utilization*, AOCS Press, Champaign, Illinois, 1995.
13. Liu, K., *Soybeans: Chemistry, Technology, and Utilization*, Aspen Publishers, Gaithersburg, Maryland, 1999.
14. List, G.R., T.L. Mounts, and A.C. Lanser, Factors Promoting the Formation of Nonhydratable Soybean Phospholipids, *J. Am. Oil Chem. Soc.* 69:443–450 (1992).
15. Van den Hout, R., G. Meerdink, and K. van't Riet, Modeling of the Inactivation Kinetics of the Trypsin Inhibitors in Soy Flour, *J. Sci. Food Agric.* 79:63–70 (1999).
16. Porter, M.A. and A.M. Jones, Variability of Soy Flour Composition *J. Am. Oil Chem. Soc.* 80(6):557–562 (2003).
17. Thomas, G.R., The Art of Soybean Meal and Hull Grinding *J. Am. Oil Chem. Soc.* 58:194–196 (1981).
18. Serna Saldivar, S.O., and L.C. Cabral, Effects of Temperature, Moisture and Residence Time on the Properties of Full-Fat Soybean Flour Produced in a Twin Extruder, *Archivos Latinoamericanos de Nutricion* 47:66–69 (1997).

19. Thakur, B.R., and P.E. Nelson, Inactivation of Lipoxygenase in Whole Soy Flour Suspension by Ultrasonic Cavitations, *Nahrung Food* 4:299–301 (1997).
20. Ferrier, L.K., and M.J. Lopez, Preparation of Full-Fat Soy Flour by Conditioning, Heating and Grinding, *J. Food Sci.* 44:1017–1031 (1979).
21. Nelson, A.L., W.B. Wijeratne, S.W. Yeh, and L.S. Wei, Dry Extrusion as an Alternative to Mechanical Expelling of Oil from Soybeans, *J. Am. Oil Chem. Soc.* 64:1341–1347 (1987).
22. Areas, J.A.G., Extrusion of Food Proteins, *Crit. Rev. Food Sci. Nutr.* 31:365–392 (1992).
23. Sevatson, E., and G.R. Huber, Extruders in the Food Industry, in *Extruders in Food Applications*, edited by M.N. Riaz, Technomics Publishing Co., Lancaster, Pennsylvania, 2000, pp. 167–204.
24. Godfrey, P., and W.F. Limpert, Soy Products as Ingredients—Farm to the Table, *Innovations in Food Technol.* Feb.:10–13 (2002).
25. Chen, M., Properties and Food Applications of Soy Flour, in *Proceedings of the World Conference on Oilseed Technology and Utilization*, edited by T.H. Applewhite, AOCS Press, Champaign, Illinois, 1993, pp. 306–310.
26. Kinsella, J.E., S. Damodaran, and B. German, Physicochemistry and Functional Properties of Oil Seed Proteins with Emphasis on Soy Proteins, in *New Protein Foods*, Vol. 5, edited by A.M. Altschul and H.L. Wilcke, Academic Press, New York, 1985, pp. 107–179.
27. Damodaran, S., Structure-Function Relationship of Food Proteins, in *Protein Functionality in Food Systems*, edited by N.S. Hettiarachchy and G.R. Ziegler, Marcel Dekker, New York, 1994, pp. 1–37.
28. Messina, M., Legumes and Soybeans: Overview of Their Nutritional Profiles and Health Effects, *Am. J. Clin. Nutr.* 70:439S–450S (1999).
29. Godfrey, P., The Power of Soy Flour: Food Applications in Wheat-Based Products, in *Proceedings of China & International Soybean Conference & Exhibition*, edited by K. Liu *et al.*, Beijing, China, 2002, pp. 152–155.
30. Food and Drug Administration, Food Labeling, Health Claims, Soy Protein, and Coronary Heart Disease, *Fed. Reg.* 57:699–733 (1999).
31. Lang, P., Functionality of Full Fat and Low Fat Soy Ingredients, presented at Soyfoods '99, Chicago, April 26–28, 1999.

Chapter 6

Soy Protein Concentrate: Technology, Properties, and Applications

Daniel Chajuss

Hayes General Technology Company, Misgav Dov, Emek Sorek 76867, Israel

The soybean [*Glycine max* (L.) Merrill] is one of the oldest crops cultivated by man. In China, where it constitutes an important source of food protein, the soybean has been used for several thousand years. From China the soybean has spread throughout a large portion of the world, and is now extensively grown in most parts of the world, partly due to its good adaptability to an extensive variety of soil and climatic conditions. Whereas the soybean was largely grown as a food crop in the Orient, its principal uses today are for the production of oil for human consumption and meal for animal feed.

The soybean is exceptionally rich in good quality functional protein, with a composition of about 40% crude protein on dry basis, determined from Kjeldahl N (organic nitrogen) multiplied by 6.25. The high-protein composition of soybean has led to the development of numerous industrial protein food ingredients such as full-fat and defatted soy flours, textured soy flour, soy protein isolates, soy protein concentrates, textured soy protein concentrate, and enzyme-treated soy protein products. Soy protein has long been regarded as one of the world's least expensive good quality available protein sources (1).

In recent years very useful and updated information has been published on industrial soybean protein products and their chemistry, technology, and utilization. (2,3). The purpose of this chapter is to expound on this information from the industry standpoint.

Soybean Proteins

Most of the protein in soy is found in storage sites called protein bodies or aleuronic grains, which are subcellular structures of 2 μm to 20 μm in diameter. The protein bodies were reported to contain about 10% nitrogen, 0.8% phosphorus, 8.5% sugar, 7% ash, and 0.5% RNA (4), and to contain approximately 4.5% lipid and 2.0% phospholipid (5). K  le (6) reported that the protein bodies are nearly 75% protein and that the globular reserve proteins make up about 80% of the soy seed protein, whereas biologically active proteins (enzymes, enzyme inhibitors, lectins, etc.) make up the remaining 20%.

The soybean storage proteins were first extracted and characterized by Osborne and Campbell in 1898. Osborne and Campbell named the extracted protein glycinin

(7). Later workers noted that this protein is heterogeneous and when subjected to ultracentrifugation gave, at pH 7.6 and 0.5 ionic strength, the fractions 2S, 7S, 11S, and 15S (8,9). Catsimopoolas (10) suggested basing the classification of soy protein components on an immunochemical reference system. Four immunochemically distinct globulins have been identified as follows: glycinin that matches the 11S globulin (not to be confused with the glycinin of Osborne and Campbell), α -conglycinin that is a part of the 2S globulin fractions, and β -conglycinin and γ -conglycinin that are part of the 7S fraction. The bulk of the native soy storage proteins are composed of glycinin (11S globulin) and β -conglycinin (7S globulin).

Although proteins of plant origin are often of lower nutritional quality than proteins of animal origin due to deficiency in one or more of the essential amino acids, soy protein has a relatively well-balanced amino acid composition, limited by sulfur-containing amino acids (11).

One method used to test protein quality is based on feeding the protein product to rats to provide the protein efficiency ratio (PER). The PER of soy protein is lower than the PER of animal proteins, but upon fortification with sulfur-containing amino acids it reaches almost the same PER level as animal proteins. As rats depend heavily on sulfur-containing amino acids, the PER underestimates the protein content for soy protein when compared with feedings of soy protein to other animal species (12). Presently a protein assay method called the protein digestibility-corrected amino acid score (PDCAAS) is employed; the quality of soy protein determined by this assay is comparable to that of animal proteins (13–15).

Besides amino acid composition, there are other factors that affect the nutritional quality of soy protein. These factors include treatments of the soy protein by heat and the means of the heat application; modification of the soy globulin structures to render them free of antigenicity, for instance, by aqueous alcohol and heat; and presence of possible antinutrients at biologically active levels within the soy protein matrix. Most of the soy proteins' antinutritional factors are destroyed by heat treatment or by aqueous alcohol extraction.

Other factors considered to affect the quality of soy protein, along with dietary qualities, are functionality, taste, shape and form, and physical conditions.

Soy Protein Concentrate

The most common industrial protein food ingredients are soy flours (full-fat or defatted, toasted or enzyme-active, and textured), soy protein isolate, and soy protein concentrate. In 2001 about 350,000 metric tons of soy protein concentrate were produced and sold worldwide. Soy protein concentrate is a purified, relatively bland protein product containing a minimum of 65% protein on a moisture-free basis (Kjeldahl N \times 6.25). It is obtained from defatted soybean flakes or flour by removal of nonprotein components. More specifically, soy protein concentrate is made under conditions where the bulk of the proteins are rendered insoluble. The sugars and other low-molecular-weight constituents are dissolved, leaving the protein and the cell wall polysaccharides.

The dissolving agents considered over the years for use in processes to produce soy protein concentrate were water leaching of heat-denatured defatted soybean flour (16), diluted-acid leaching at an isoelectric pH of 4.5 (17), and aqueous alcohol (18).

Acid-washed soy protein concentrate was available commercially in the early 1950s. The acid-washed concentrate has better applicability and taste than toasted soy flour, better stability and taste than enzyme-active non-toasted soy flour, and is lower in cost than soy protein isolates, serving in applications where the lower protein content is of less importance.

The advantages of acid-washed soy protein concentrate are the following:

- No inflammable solvents are used.
- Only slightly denatured and relatively soluble product is obtainable.

The disadvantages of acid-washed soy protein concentrate are the following:

- It cannot be converted into textured products.
- The process creates a high amount of liquid effluents.
- Lower yields are obtained than in the aqueous alcohol wash technology.
- It is of lower nutritional quality, containing antigenic proteins as the 2S, glycinin, and β -conglycinin.
- It has low salt tolerance in meat systems.
- Flavor of product often is soapy.
- A large amount of water drying, using spray drying systems, is required.
- Stainless steel equipment and frequent cleaning using a “clean in place” (CIP) system are required.

These disadvantages have led to a transition to the predominant use of an aqueous alcohol-washed (“traditional”) soy protein concentrate production system.

Aqueous alcohol-washed concentrate was introduced commercially in the early 1960s. Central Soya’s Chemurgy Division in the United States developed an immersion aqueous alcohol-extraction system and at about the same time Chajuss of Hayes Company in Israel introduced a continuous counter-current aqueous alcohol wash system. The producers of traditional-type concentrate generally use this system today. The production flow is shown in [Figure 6.1](#).

The aqueous alcohol wash process is based on the ability of aqueous solutions of lower aliphatic alcohols (methanol, ethanol, and isopropanol) to extract the soluble fraction of defatted soy flakes without solubilizing its proteins. The aqueous alcohol-washed soy protein concentrate is manufactured industrially by extracting defatted non-toasted soybean flakes having NSI (Nitrogen Solubility Index) of 50 to 70 with 60% to 70% warm aqueous ethanol, or when warranted with warm aqueous isopropanol (IPA), depending on the availability and the relative prices of ethanol and isopropanol. The aqueous alcohol-washed soy protein concentrate is termed

“traditional concentrate” and the dealcoholized aqueous alcohol-soluble material is termed “soy molasses” (19). Table 6.1 provides a typical gross analysis of traditional aqueous alcohol-washed soy protein concentrate.

The advantages of the traditional soy protein concentrate are the following:

- Simple, efficient, and cost-effective continuous operation with low operating costs.
- High yields are obtainable.
- No wastes or effluents are generated and no special water or waste treatments are required.
- The obtained soy protein concentrate can be textured into very high quality bland textured protein products by a simple low-cost technology.
- The obtained soy protein concentrate can be converted into highly functional and soluble products of high solubility, good emulsification properties, and high water and fat absorption.
- It is free of estrogenic activity (isoflavones), and thus suitable for infant formulas.
- The product is relatively bland, free of “beany” flavors and tastes in particular after being converted (“refolded”) into functional types of soy protein concentrate.

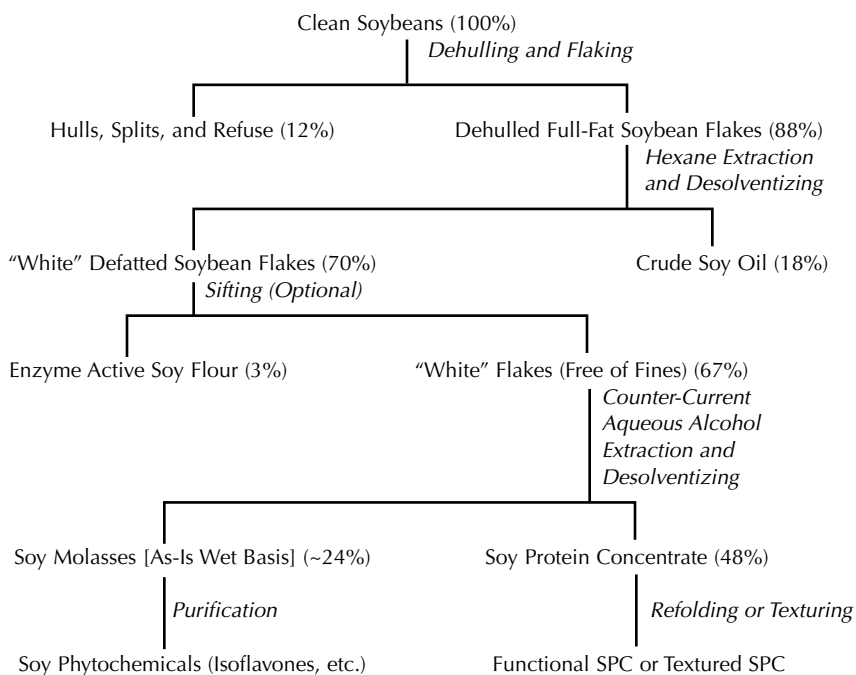


Figure 6.1. Typical material flow: Soy protein concentrate—traditional alcohol wash system.

- It has high salt tolerance in meat systems.
- No need to use CIP system due to use of alcohol in the system.

The disadvantage of aqueous alcohol washed soy protein concentrate is the following:

- Use of inflammable and highly explosive solvent (aqueous alcohol) in the process necessitates explosion-proof equipment and extra safety precautions while in operation.

TABLE 6.1

Typical Analysis of "Traditional" Aqueous Alcohol-washed Soy Protein Concentrates

Constituents				Composition (%)	
Moisture				6–10	
Protein (N × 6.25; dry basis)				68–72	
Typical Amino Acid Profile					
Amino Acid g/16 g N			Amino Acid g/16 g N		
Isoleucine*	4.8	Arginine	7.6		
Leucine*	7.8	Aspartic acid	11.5		
Lysine*	6.3	Glutamic acid	19.5		
Methionine*	1.4	Proline	5.2		
Phenylalanine*	5.3	Glycine	4.4		
Threonine*	4.2	Alanine	4.4		
Tryptophan*	1.5	Cysteine	1.6		
Valine*	5.0	Tyrosine	3.9		
Histidine	2.7	Serine	5.6		
Fat (ether extract)				0.5–1.0	
Crude fiber				3–5	
Minerals (Ash), Total				4–6	
Potassium	1.98	Magnesium	0.25		
Phosphorous	0.66	Silicone	0.05		
Sulfur	0.41	Iron	0.01		
Calcium	0.25	Sodium	0.01		
Carbohydrates (mainly pectic-like acidic polysaccharides), Total				16–20	
Microbial	Total Plate Count	< 5,000 per gram			
	Salmonella	Negative in 25 grams			
	<i>E. coli</i>	Negative in 1 gram			
	Yeast and mold count	< 100 per gram			
Other characteristics					
Flavor Bland; PER 2; NSI 6–12; Color Off-white					
Substantially free of antigenic proteins (2S; glycinin, and β-conglycinin)					
Essentially free of enzymatic and anti-enzymatic activities					
Shelf life at least one year when stored in a dry place, preferably below 28°C at a relative humidity of 65% or less					

* Essential Amino Acid

World production of soy protein concentrate is primarily concentrated in the hands of a few manufacturers. Aqueous alcohol washed concentrates are manufactured by Archer Daniels Midland (ADM) in the United States and The Netherlands; by Central Soya in the United States, France, and Denmark; and by Solbar Hatzor (previously Hayes Ashdod) in Israel. Acid-washed concentrate is made mainly by Ceval Alimentos in Brazil, by ADM in the United States, and in small quantities by other manufacturers elsewhere.

Approximately 400,000 metric tons of the soy protein concentrate currently produced is manufactured by the counter-current aqueous alcohol wash system. Of these, roughly 25% are further converted to functional soy protein concentrate and roughly 20% are textured. Approximately 20,000 metric tons (about 6%) are produced by an acidified water extraction. Soy protein concentrate production by water leaching of denatured defatted soybean flour was attempted for a short period in the late 1960s by Swift Company in the United States but has not been produced since.

Properties and Applications

Roughly 60% to 70% of the soy protein concentrate produced is used for human consumption, the rest being used for milk replacers for calves and piglets, fish feeds, and pet foods. A small amount is used for nonfood, nonfeed applications, for example, for paper coatings.

Considerable work was done on the nutritional aptness of aqueous alcohol-extracted soy protein concentrate, mainly in relation to its utilization in milk replacers for calves and as an ingredient in fish food (20–22). Studies on human volunteers confirmed digestibility of aqueous alcohol-washed soy protein concentrate to be comparable to that of animal proteins (23,24). A long-term metabolic study was conducted with volunteer subjects wherein during a three-month test period the participants received a diet in which aqueous alcohol-washed soy protein concentrates (“traditional” and “functional”) were the only protein source at a level similar to the FAO-recommended minimum level of high-quality protein. The results showed that the volunteers were in good health during the entire test period and that the soy protein concentrate has the same protein quality as animal proteins. It was further observed that aqueous alcohol-washed soy protein concentrates are well tolerated and that their immunological activity is very low (25). Hot aqueous alcohol wash removes, denatures, or modifies biologically active constituents of the soy protein concentrate to render them inactive. The immunologically active soy proteins and the soy proteolytic enzyme inhibitors [Kunitz trypsin inhibitor and the Bowman-Birk trypsin and chymotrypsin inhibitors (BBI)] are considered the main adverse components, especially for calves’ milk replacers and fish feeds; the aqueous alcohol wash removes, destroys, or modifies these constituents. Tests indicative of the presence of specific antigens in soy protein products by hemagglutination inhibition assay (26) and competitive inhibition ELISA for quantification of residual undenatured glycinin and β -conglycinin based on the methods and reagents described by Voller and cowork-

ers (27), as well as tests for trypsin inhibitor activity, are commonly used by the industry to ensure the quality of the soy protein concentrate material to be used in particular for feed purposes.

Traditional soy protein concentrate is a valuable food component and a functional protein ingredient. Soy protein concentrates replace meat, fish, poultry, and milk proteins with economic benefit in industrial meat processing and are used in vegetarian meat alternatives. Soy protein concentrates are also incorporated in formulations for calves' and piglets' milk replacers, in pet foods, and in special feed-stuffs such as fish feeds to obtain less "fishy" and bland fish meats and feeds for mink and other fur animals. Soy protein concentrate is usefully applied in bakery products, in dietetic foods, and in infant formulas. The uses of soy protein concentrate are summarized in Table 6.2.

TABLE 6.2
Applications of Traditional Soy Protein Concentrate

Soy Product	Typical Uses of Soy
Textured soy protein	Makes high-quality textured soy protein concentrate products for partial or complete replacement of meat, fish, and poultry in processed food products, and for non-meat alternatives and analogs
"Functional concentrates"	Make "functional" soy protein concentrates having high water and fat absorption, high dispersability, and tailored functionality that can replace soy protein isolates and caseinates with improved functionality and cost advantage
Minced meat products	In sausages, hamburgers, luncheon meats, meat loaves, etc., as high-quality extenders and meat replacers; in the meat processing industry, to improve quality and lower manufacturing costs
Fish products	In fish balls, fish pastes, fish fingers, etc., to improve quality and lower costs; in canned tuna and other solid fish products to ensure texture, volume, and juiciness
Bakery products	In breads, crackers, pastry, fillers, etc.
Dairy products	In cheeses, coffee whiteners, ice creams, and frozen desserts
Breakfast cereals	Add protein nutrition to breakfast cereals and to improve breakfast bars
Dietetic foods	Hypoallergenic foods, baby formulas, vegetarian foods, slimming diets, health foods, high-protein sports formulas, etc.
Feed starters and milk replacers	Replace skim milk powder for rearing calves, piglets and other suckling animals with all-around economic advantage
Pet foods and special animal diets	Highly acceptable and concentrated protein source with well balanced amino acid ratio

However, traditional alcohol-washed concentrate has low protein solubility (NSI values 6–12) due to denaturation of the protein by the aqueous alcohol. In contrast, alcohol-washed concentrate retains much of the protein functional properties (water binding, oil binding, slurry viscosity, emulsification power, etc.) despite its low protein solubility.

The protein solubility, slurry viscosity, dispersibility, emulsification properties, water absorption, water binding capacity, and oil binding capacity are enhanced commercially by the industry in several ways, from a simple addition of gums and other additives in a process that produces a “pseudo” functional concentrate to more laborious techniques of protein “refolding.” Functional “refolded” soy protein concentrates were initially made industrially according to the teachings of Howard and co-workers (28) by adding sodium, potassium, and/or calcium hydroxides; heating; homogenizing; neutralization; and drying. Chajuss (29) introduced ammonia as an easily stripped alkalizing agent. Presently improved technologies, based on the above methods are used commercially. These include pre-washing of the traditional soy protein concentrate to remove non-protein solubles; high temperature steam treatment; increased holding time before drying; etc. The functional soy protein can be further converted to particular “functional” concentrates (fully soluble concentrate and high viscosity material, etc.).

Enhanced functional properties of a protein material are measured by the ability of protein material to hold oil or fat and water, to emulsify the same, and to form products having a firm gel-like consistency upon heating and cooling. A customary method used by the industry for determining the functional properties of protein products is as follows: Five to seven parts of refined vegetable oil (e.g., corn oil) and half that amount of water (2.5 to 3.5 parts) are well mixed in a blender at maximum speed for 5 minutes. One part of the tested protein material and an additional half amount of water (2.5 to 3.5 parts) are added and mixing is continued for an additional 10 minutes. The mixture is quickly heated to 90°C, poured into cups and cooled overnight (in a refrigerator) to a temperature of 5°C. Formation of a homogeneous product having a firm consistency without separation of oil or water is indicative of a highly functional (“1:5:5”) to a very highly functional (“1:7:7”) protein product (29).

Approximately 25% of the world soy protein concentrate produced is converted into more functional and soluble protein concentrates. These concentrates offer an economic replacement of soy protein isolates, casein, and caseinates.

The major core utilization of soy protein concentrate is in the meat, fish, and poultry processing industries and in calves’ milk replacers. The traditional soy protein concentrate is mainly used as a protein-enriching source, to prevent cooking losses and to impart water and fat absorption. It is commonly used at levels of about 3–6% of the final product (as dry soy protein concentrate). The textured soy protein concentrates are used mainly to impart hydration texture and structure to meat trimmings and mechanically deboned meat, poultry, and fish, as well as to economically replace ground meat, fish, or poultry. Textured soy protein concentrates are used at levels of up to 10% of the final product (as dry textured soy protein concentrate). The functional soy protein concentrate is used to make emulsions, to absorb and

hold moisture and fat, to make firm products, and to act as a protein stabilizer in fat, rind, and meat emulsions and in “brines” used for tumbling or injection. Functional soy protein concentrate is commonly used at levels of 1–4% of the final product (as dry functional soy protein concentrate). Some representative applications of soy protein concentrate in processed meat are presented in Tables 6.3, 6.4, and 6.5.

TABLE 6.3
Beef Burger with Textured and Functional Soy Protein Concentrates

Ingredients	%
Beef trimmings 40% fat	58.00
Textured soy protein concentrate (small granules or flakes)	9.00
Functional soy protein concentrate	2.00
Water	28.00
Salt	0.80
Black pepper	0.10
Spice	2.10
Total	100.00

Procedure:

1. Hydrate the textured protein concentrate with cold water for 15–20 minutes in a ribbon blender.
2. Place the meat trimmings, hydrated textured soy protein concentrate, salt, pepper, spice, and functional concentrate and mix for 3–5 minutes until a uniform mix is reached.
3. Grind the mixture through a 3–4 mm plate.
4. Form burger patties.

TABLE 6.4
Cured Ham with Functional Soy Protein Concentrate

Ingredients	%
Lean pork (ham) muscle cuts	64.00
Brine	
consisting of:	
Water	27.40
Functional soy protein concentrate	1.20
Dextrose	2.80
Salt	2.25
Corn syrup solids	1.92
Phosphates	0.30
Curing nitrite salt (6.25% nitrite)	0.10
Sodium erythorbate	0.03
Total Brine	36.00
Total	100.00

Procedure:

1. Prepare brine and inject it with multi-needle injector into the muscle cuts several times until the brine is well absorbed
2. Chop the ham cuts with the added brine to about 10 mm pieces.
3. Vacuum tumble the injected and chopped muscles cuts until the brine absorption is completed
4. Stuff and cook.

In calves' milk replacer formulas fine-milled traditional soy protein concentrate is used as a low-cost replacement of milk proteins; typically, a mixture of about 48% soy protein concentrate, 46% sweet whey powder, and 6% fat will substitute in any ratio the skim milk powder in calves' milk replacer formulas with comparable availability of protein and energy.

It is generally accepted nowadays that soy-containing foods are healthy. The U.S. Food and Drug Administration (FDA) authorized use of health claims about the role of soy protein in reducing the risk of coronary heart disease (CHD) on labeling of foods containing soy protein (30). This rule is based on the FDA's conclusion that foods containing soy protein included in a diet low in saturated fat and cholesterol may reduce the risk of CHD by lowering blood cholesterol levels.

Coronary heart disease, one of the most common and serious forms of cardiovascular disease, is a major public health concern because, for example, it causes more deaths in the United States than any other disease. Risk factors for CHD include high total cholesterol levels and high levels of low-density lipoprotein (LDL) cholesterol. The FDA-approved health claim is based on evidence that including soy protein in a diet low in saturated fat and cholesterol may also help to reduce the risk of CHD. Recent clinical trials have shown that consumption of soy protein, as compared to other proteins such as those from milk or meat, can lower total and LDL cholesterol levels. Jenkins and coworkers (31) reported that no significant differences were observed between high-isoflavone and low-isoflavone soy diets. The soy diets (compared to non-soy diets) resulted in significantly lower total cholesterol, lower estimated coronary artery disease (CAD) risk, and lower ratios of total cholesterol to HDL cholesterol, LDL cholesterol to HDL cholesterol, and apolipoprotein B to apolipoprotein A-I. The calculated CAD risk was significantly lower with the soy diets, reduced by $10.1 \pm 2.7\%$.

TABLE 6.5
Beef Roll with Traditional Soy Protein Concentrate

Ingredients	%
Beef trimmings 25% fat	77.30
Soy protein concentrate	5.00
Water	15.00
Phosphates	0.30
Salt	1.80
Spice	0.60
Total	100.00

Procedure:

1. Grind the beef trimmings 10 mm to 25 mm.
2. To the ground beef trimmings add spice, salt, and phosphates and mix well.
3. Add the soy protein concentrate and water simultaneously while mixing.
4. Continue mixing for 5 minutes under vacuum.
5. Stuff and cook to an internal temperature of 68°C.

Prospects

The market for soy protein concentrate has been steadily growing in recent years. Changes in public policies and regulations, consumers' trends towards vegetarianism and concerns about bovine spongiform encephalopathy (BSE), and climbing prices of dairy proteins and other protein sources have led to a large demand for vegetable proteins in general and for soy protein concentrate in particular. The demand for high-quality, low-cost protein as alternatives or substitutes for meat is well manifested in developed as well as in developing nations and is expected to expand. Textured and functional soy protein concentrates typically having a large and growing market share.

The ruling of the FDA that allows labeling of soy-based products to indicate that a 25-gram intake of soy protein daily, combined with a diet low in saturated fat and cholesterol, could help prevent heart disease (30), may further promote an increase of soy protein concentrate consumption, helping soy to find a new and growing niche as a nutritive functional ingredient in foods, in particular in foods labeled "dietetic foods," "nutritional bars," and "health foods."

The potential utilization of soy protein concentrate as meat extenders and alternatives in the meat processing industry; in the food processing industry in general; as an ingredient of milk replacers and starters for young suckling animals, particularly calves and piglets; as an ingredient in fish feeds; and as a healthy food ingredient in human diets is estimated to reach as much as a million tons per year within the next decade.

The extent to which this market potential can be achieved depends upon several factors including the availability of funds and accessibility of technology and know-how, the pace of development of the food manufacturing industry, monetary and other government policies, consumer acceptance of the formulated products, and the availability of local dairy and other alternative proteins.

References

1. Campbell, M.F., Processing and Product Characteristics for Textured Soy Flours, Concentrates and Isolates, *J. Am. Oil Chem. Soc.* 58:336–339 (1980).
2. Liu, K.S., *Soybeans Chemistry, Technology and Utilization*, Aspen Publishers, Gaithersburg, Maryland, 1999.
3. Endress, J.G., *Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization*, AOCS Press, Champaign, Illinois, 2001.
4. Saio, K., and T. Wantabe, Preliminary Investigation on Protein Bodies of Soybean Seeds, *Agr. Biol. Chem.* 30:1133–1138 (1966).
5. Boatright, W.L., and H.E. Snyder, Soybean Protein Bodies: Phospholipids and Phospholipase D Activity, *J. Am. Oil Chem. Soc.* 70:623–628 (1993).
6. Køle, B., *Karakterisering, Varmebehandling og næringsværdi af Sojebønnerproteiner* [Characterization, Heat Treatment and Nutrition Qualities of Soybean Proteins], Ph.D. Thesis, Technical University, Denmark, 1973.
7. Osborne, T.B., and G.P. Campbell, Proteids of the Soybean, *J. Am. Chem. Soc.* 20:419–428 (1898).

8. Naismith, W.E.P., Ultracentrifuge Studies on Soybean Protein, *Biochim. Biophys. Acta* 16:203–210 (1955).
9. Wolf, W.J., and D.R. Briggs, Ultracentrifugal Investigation of the Effect of Neutral Salts on the Extraction of Soybean Proteins, *Arch. Biochem. Biophys.* 63:40–49 (1956).
10. Catsimopoulos, N., A Note on the Proposal of an Immunochemical System of Reference and Nomenclature for the Major Soybean Globulins, *Cereal Chem.* 46:369–372 (1969).
11. Circle, S.J., and A.K. Smith, Processing Soy Flours, Protein Concentrates and Protein Isolates, in *Soybeans: Chemistry and Technology, Vol. I. Proteins*, edited by A.K. Smith and S.J. Circle, AVI Publishing Company, Westport, Connecticut, 1978, pp. 294–338.
12. Bender, A.E., Evaluation of Protein Quality: Methodological Considerations, *Proc. Nutr. Soc.* 41:267–276 (1982).
13. Sarwar, G., and F.E. McDonough, Evaluation of Protein Digestibility-Corrected Amino Acid Score Method for Assessing Protein Quality of Foods, *J. Assoc. Off. Anal. Chem.* 73:347–356 (1990).
14. Schaafsma, G., The Protein Digestibility-Corrected Amino Acid Score, *J. Nutr.* 130:1865S–1867S (2000).
15. Food and Agriculture Organization of the World Health Organization, *Protein Quality Evaluation, FAO/WHO Nutrition Meetings*, Report Series 51, Author, Rome, Italy (1990).
16. McAnelly, J.K., Method for Producing a Soybean Protein Product and the Resulting Product, U.S. Patent 3,142,571, July 28, 1964.
17. Sair, L., Proteinaceous Soy Composition and Method of Preparing, U.S. Patent 2,881,076, April 7, 1959.
18. Mustakas, G.C., L.D. Kirk, and E.L. Griffin, Flash Desolventizing of Defatted Soybean Meals Washed with Aqueous Alcohol to Yield a High Protein Product, *J. Am. Oil Chem. Soc.* 39:222–226 (1962).
19. Chajuss, E.M., and D. Chajuss, Process for the Production of Molasses-like Syrup, Israel Patent 19168, May 6, 1963.
20. Berge, G.M., B. Grisdale-Helland, and S.J. Helland, Soy Protein Concentrate in Diets for Atlantic Halibut (*Hippoglossus hippoglossus*), *Aquaculture* 178:139–148 (1999).
21. Erickson, P.S., D.J. Schauff, and M.R. Murphy, Diet Digestibility and Growth of Holstein Calves Fed Acidified Milk Replacers Containing Soy Protein Concentrate, *J. Dairy Sci.* 72:1528–1533 (1989).
22. Mambrini, M., A.J. Roem, J.P. Carvèdi, J.P. Lallès, and S.J. Kaushik, Effects of Replacing Fish Meal with Soy Protein Concentrate and of DL-Methionine Supplementation in High-Energy, Extruded Diets on the Growth and Nutrient Utilization of Rainbow Trout, *Oncorhynchus mykiss*, *J. Anim. Sci.* 77:2990–2999 (1999).
23. Istfan, N., E. Murray, M. Janghorbani, and V.R. Young, An Evaluation of the Nutritional Value of a Soy Protein Concentrate in Young Adult Men Using the Short-Term N-Balance Method, *J. Nutr.* 113:2516–2523 (1983).
24. Istfan, N., E. Murray, M. Janghorbani, W.J. Evans, and V.R. Young, The Nutritional Value of a Soy Protein Concentrate (STAPRO-3200) for Long-Term Protein Nutritional Maintenance in Young Men, *J. Nutr.* 113:2524–2534 (1983).
25. Beer, W.H., E. Murray, S.H. Oh, H.E. Pedersen, R.R. Wolfe, and V.R. Young, A Long-Term Metabolic Study to Assess the Nutritional Value of and Immunological Tolerance to Two Soy-Protein Concentrates in Adult Humans, *Am. J. Clin. Nutr.* 50:997–1007 (1989).

26. Pederson, H.C.E., *Studies of Soybean Protein Intolerance in the Preruminant Calf*, Ph.D. Thesis, University of Reading, Reading, Berkshire, England (1986).
27. Voller, A., D.E. Bidwell, and A. Barlett, Enzyme Immunoassays in Diagnostic Medicine, *Bull. World Health Org.* 53:561–566 (1976).
28. Howard, P.A., M.F. Campbell, and D.T. Zollinger, Water-soluble vegetable protein aggregates, U.S. Patent 4,234,620, November 18, 1980.
29. Chajuss, D., Process for Enhancing Some Functional Properties of Proteinaceous Material; U.S. Patent 5,210,184, May 11, 1993.
30. U.S. Federal Register [Rules and Regulations] 64(206), October 26, 1999.
31. Jenkins, D.J., C.W. Kendall, C.J. Jackson, P.W. Connelly, T. Parker, D. Faulkner, D. Vidgen, S.C. Cunnane, L.A. Leiter, and R.G. Josse, Effects of High- and Low-Isoflavone Soyfoods on Blood Lipids, Oxidized LDL, Homocysteine, and Blood Pressure in Hyperlipidemic Men and Women, *Am. J. Clin. Nutr.* 76:365–372. (2002).

Chapter 7

Isolated Soy Protein: Technology, Properties, and Applications

William Russell Egbert

Archer Daniels Midland Company, Decatur, IL 62526

The typical composition of the soybean is 18% oil, 38% protein, 15% insoluble carbohydrate (dietary fiber), 15% soluble carbohydrate (sucrose, stachyose, raffinose, and others), and 14% moisture, ash, and other. The soybeans are cracked to remove the hull and rolled into full-fat flakes. The rolling process disrupts the oil cell, which facilitates solvent extraction of the oil. After the oil has been extracted, the solvent is removed and the flakes are dried, which creates defatted soy flakes. The defatted flakes can then be ground to produce soy flour, sized to produce soy grits, or texturized to produce TVP®. The defatted flakes can be further processed to produce soy protein concentrate and isolated soy protein. This is accomplished by the removal of the carbohydrate components of the soybean followed by drying.

Soy proteins are generally classified into the following three groups: soy flours, soy protein concentrates, and isolated soy proteins, with minimum protein contents of 50%, 65%, and 90% (dry basis), respectively. Soy flours are sold as either fine powders or grits with a particle size ranging from approximately 0.2 to 3 mm. These products can be manufactured by using minimal heat to maintain the inherent enzyme activity of the soybean or by lightly to highly toasting to reduce or eliminate the active enzymes and improve product flavor. Soy flours and grits have been used traditionally as an ingredient in the bakery industry.

Soy protein concentrates are traditionally manufactured by using aqueous alcohol to remove the soluble sugars from the defatted soy flakes (soy flour). This process results in a protein with low solubility and a product that can absorb water but lacks the ability to gel or to emulsify fat. Traditional alcohol-washed concentrates are used for protein fortification of foods as well as in the manufacture of textured soy protein concentrates. Functional soy protein concentrates can be produced from alcohol-washed concentrate by using heat and homogenization followed by spray drying, or produced by using a water-wash process at an acid pH to remove the soluble sugars followed by neutralization, thermal processing, homogenization, and spray drying. Functional soy protein concentrates bind water, emulsify fat, and form a gel upon heating. Functional soy protein concentrates are widely used in the meat industry to bind water and emulsify fat. These proteins are also effective in stabilizing high fat soups and sauces.

Textured or structured soy proteins can be made from soy flour, soy protein concentrate, or isolated soy protein. TVP® is manufactured through thermoplastic ex-

trusion of soy flour under moist heat and high pressure. There are many sizes, shapes, colors, and flavors of TVP®; bacon-colored and -flavored products are some of the most popular products. Textured soy protein concentrate is produced from soy protein concentrate powders by using manufacturing technology similar to that for TVP®. Unique textured protein products can be produced by using combinations of soy protein or other powdered protein ingredients such as wheat gluten in combination with various carbohydrate sources (e.g., starches). The products that contain wheat gluten are used more widely in vegetarian applications to simulate ground meats or meat chunks and strips. Textured products manufactured by thermoplastic extrusion technology are distributed throughout the world in the dry form. These products are hydrated in water or flavored solutions before use in processed meat products, vegetarian analogs, or used alone in other finished food products to simulate meat. Spun-fiber technology can be used to produce a fibrous textured protein from isolated soy protein with a structure closely resembling meat fibers. These products can also be colored or flavored to obtain the desired finished product. The disadvantages to spun-fiber products are the high cost of manufacture coupled with the high cost of product distribution over long distances while either refrigerated or frozen.

Isolated soy proteins are manufactured from defatted soy flakes by separation of the soy protein from both the soluble and the insoluble carbohydrate fractions of the soybean. This chapter will focus on the development of the technology currently used in the industry to manufacture isolated soy protein, the functional characteristics of these proteins, and the use of isolates in food applications. The following section will cover the development of the technology for the production of isolated soy proteins.

Technological Development

Isolated soy protein development has a history that dates back more than 60 years. Early development was focused on the production of isolated soy proteins for the manufacture of paper coating and composite fiber development. Cone and Brown (1) first disclosed the treatment of soy and other seed proteins by the use of aqueous solutions of caustic alkali from lime or with salts. They concluded that the separation could be completed by settling or centrifugation. This technology focused on the development of isolated soy proteins for the paper coating industries. In 1941, Julian and Engstrom (2) patented technology that used hot-acid isoelectric separation for the production of films and coatings. By the late 1940s, patents were issued for the production of isolated soy proteins by the use of alkaline separation with centrifugation followed by acid precipitation of the protein to remove other water-soluble materials including soluble sugars (3,4). Again, these patents were focused on commercial nonfood uses for isolated soy proteins.

The first technological developments of isolated soy proteins for use in food applications appear to be in the late 1940s and early 1950s by the Central Soya

Company, Inc (5–7). The focal point of these developments was the production of albumen-like whipping agents to replace egg white protein. They included enzymatic modification of the isolated soy proteins to reduce viscosity and improve whipping characteristics of the protein through the use of pepsin treatment. Sair and Rathman (6) initiated the established parameters for the alkaline separation and acid precipitation processes in the production of isolated soy proteins. Both pH and temperature parameters were refined. Extraction pH of 8–10.5 and temperatures of 22–25°C were found to be most beneficial for alkaline separation of the insoluble fractions of the defatted soybean meal from the soluble carbohydrate and protein fractions. Acid precipitation was completed at about pH 4.2. Circle and colleagues (8) patented technology in 1959 that focused on yield improvement as well as improvements in color and taste of isolated soy proteins. This technology incorporated the use of sodium hydroxide for alkaline extraction at temperatures of 55–75°C and a pH of 6–8. Anson and Pader (9) suggested that alkaline extraction technology using 0.002–0.004 M calcium hydroxide at 60°C would produce a good flavored and colored isolated soy protein that would be sufficiently clean to be approved as an edible protein source. Protein extractions within this calcium hydroxide molarity range would provide an extraction pH of 6.7 to 7.2. Isolated soy proteins produced using this method should have good gelling characteristics and work well in simulated meat and meat products. Calcium hydroxide continues to be used in the front-end alkaline extraction process of some commercially produced isolated soy proteins.

The processes for improving the solubility, gelling, and emulsification characteristics of soy protein extracts were further refined by Sair (10) with modifications to the isolated soy protein process after alkaline extraction and acid precipitation. Sair suggested pH adjustment of the alkaline-extracted and acid-precipitated soy protein to above 6.0 in the presence of suspending water. This neutralized extract was heated to a temperature of 50–85°C and then dried. The resulting isolated soy protein powder had improved solubility, gelling, and emulsification properties. Gelling properties appeared to improve with increased heat treatment. This technology was further refined by Hawley and colleagues (11) through the use of jet cooking and flash cooling. Jet cooking is a process in which the extracted protein slurries are heated almost instantaneously under pressure by the use of steam-injection nozzles. These steam-injection systems are commonly referred to in the manufacturing industries as “jet cookers.” This results in rapid temperature elevation as well as severe physical disruption of the protein matrix. Flash cooling is the process of discharging the pressurized heated slurry into a lower pressure zone, typically under vacuum. This sudden drop in pressure results in the instantaneous reduction in temperature as well as the release of volatile unwanted flavor and odor components. The Hawley and colleagues (11) technology consisted of neutralizing an isolated soy protein slurry to a pH of 5.7 to 7.5 at 5–17% solids, jet cooking that slurry to temperatures of 105–205°C, followed by flash cooling the protein slurry to below 100°C. This process was advantageous in that the resultant products had better fla-

vor while retaining the solubility and other functional characteristics of the isolated soy protein. This technology continues to play a major role in the commercial production of isolated soy proteins in the marketplace today.

The production of isolated soy proteins by the use of ultrafiltration membranes was patented by Frazeur and Huston (12), of the Grain Processing Corporation, in 1973. This process used conventional alkaline extraction of the insoluble fractions of homogenized defatted soy flakes via centrifugation. The soy protein and soluble sugar fractions were then further separated by the use of membrane-separation technology. This technology is based on the ability of naturally occurring salts, soluble carbohydrates, and nitrogenous materials of small molecular size to rapidly pass through a membrane while larger molecular size proteins are retained. This retained protein is then further processed and spray dried. The ultrafiltration process captures both isoelectric soluble and insoluble proteins, which results in higher protein yields. These proteins are reported to have improved nutritional advantage as a result of high sulfur-containing amino acid recovery as well as improved color, flavor, and water-holding and fat-emulsification properties. Several commercial plants have been built to produce isolated soy protein and functional soy protein concentrates based on this technological development. These plants have faced continual microbial issues as well as inherent problems with the membrane-separation systems.

Gomi and colleagues (13,14), of the Ajinomoto Company, developed technology for the production of isolated soy protein from denatured soybean flake material. This technology allows for the production of high-quality isolated soy proteins from very low solubility alcohol-extracted soy protein concentrates. The alcohol-washing process used to remove soluble sugars from defatted soybean meal also removes some of the yellow pigments associated with the soy protein as well as characteristic "beany flavor" components and objectionable bitter components. This alcohol-washing process at the same time denatures the protein and significantly reduces the protein solubility. The Gomi and colleagues (13,14) technology restores the solubility of this denatured protein. This technology involves slurring the alcohol-washed soy protein concentrate flakes with water at a flake-to-water ratio of up to 1 to 15, but preferably between a ratio of 1 to 7 and 1 to 12. The pH of the slurry is adjusted within the pH range of 6.5 to 9.0 and held under agitation for a minimum of 5 minutes, followed by rapid heating (preferably jet cooking) of the slurry to a temperature of 110–140°C and holding the slurry at this elevated temperature for 2 seconds to 3 minutes. The heating process is followed by rapid chilling of the slurry under vacuum, flash cooling. This process results in the production of a soluble protein material with an NSI (Nitrogen Solubility Index, a measurement of protein water solubility) of greater than 70%. This slurry is then centrifuged to remove the insoluble fractions. The soluble protein fraction is precipitated by adjusting the pH to the isoelectric point and further centrifuged to remove any of the residual soluble sugar components. This protein slurry is neutralized, heat treated, and spray dried. The resultant isolated soy proteins have improved color and flavor, enhanced

solubility in both water and sodium chloride solutions, and increased emulsification properties (13).

Walsh (15) patented a process for improving the whiteness of isolated soy proteins. This process involved heating protein precipitates to a temperature of between 45 and 65°C, preferably between 55 and 58°C, and concentrating the precipitates to a solids content of about 44%. This process is followed by resuspending the solids in water, neutralization, jet cooking, flash cooling, and spray drying.

Through these technological developments commercial isolated soy protein products have evolved over the past 60 years to provide products to the food industry that are bland in flavor and light in color with a wide range of functional characteristics. [Figure 7.1](#) illustrates the general processing schemes used in the production of the isolated soy proteins found commercially available in the marketplace today, including both water-washing and alcohol-washing processes. These processing schemes incorporate many of the technological advancements discussed earlier in this chapter. Due to the increasing demand for cleaner-flavored, lighter-colored, and more-functional isolated soy proteins, the technology will continue to be refined to meet the needs of the consuming public.

Functional Properties

Isolated soy proteins are probably the most versatile of the soy proteins and thus find use in a broad range of food products. These high-protein, spray-dried products are typically light in color and bland in flavor. The functional properties of isolated soy proteins can vary dramatically. Functionality is determined, in large part, by the specific processing parameters used for the manufacture of a given isolated soy protein. Heat, homogenization, and pH are three factors that greatly influence the functional characteristics of the finished isolated soy proteins. It is essential that product developers have a good understanding of the specific desired characteristics required in the finished food product so that the appropriate isolated soy protein can be selected for the particular application. Gelation, emulsification, viscosity, water binding, and dispersibility are important functional characteristics associated with isolated soy proteins and will be discussed in further detail in this chapter.

Product viscosity and dispersibility are important in a wide range of beverage applications. Enzyme modification is used to produce very low viscosity isolated soy protein for production of high-protein beverages and infant formula, and lecithination and agglomeration are used to improve the dispersion characteristics of an isolated soy protein in a powdered beverage application. Viscosity and gelation properties are critical in the manufacture of soy yogurt, sour cream, and soft cheese. In cream soups and high fat sauces, emulsification and viscosity are important to ensure the stability and texture of the finished products. Processed meat and meat analog applications require isolated soy proteins with good emulsification and gelation properties.

Other functional characteristics that differentiate isolated soy proteins are foaming or whipping properties, density, and solubility. Improper selection of an isolated soy protein for a given application often ends in frustrated product development

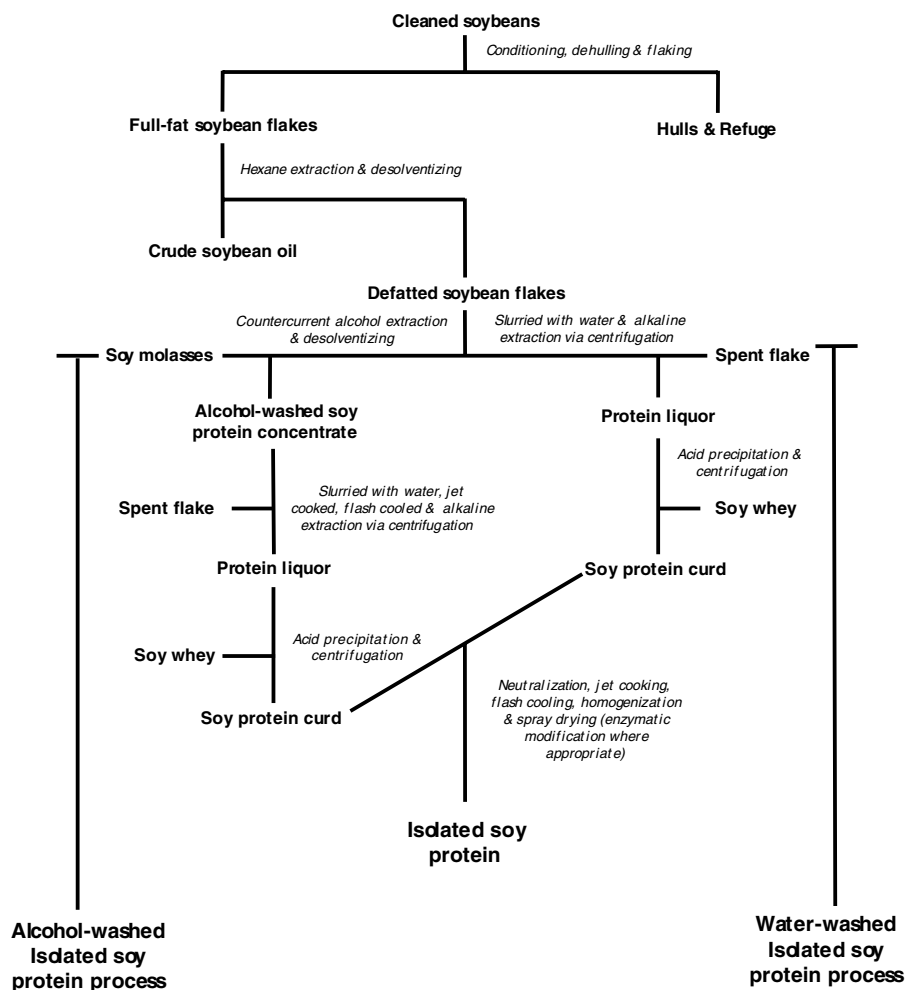


Figure 7.1. Processing schematic for water-washed and alcohol-washed isolated soy proteins.

efforts, product failure during manufacture, or unsuccessful penetration into the marketplace.

Solubility

Solubility for soy proteins is a measurement of the amount of protein that remains in suspension after centrifugation. This is not a true solution, in the terms of solubility, but is the accepted terminology used in protein literature and throughout the protein industry for discussions related to protein solubility. The soy protein industry

uses two methods for determining solubility: Protein Dispersibility Index (PDI), and Nitrogen Solubility Index (NSI). Both of these are official methods of the American Oil Chemists' Society. NSI is the method of choice for determining the solubility of isolated soy proteins as well as functional soy protein concentrates. Soy proteins have the lowest solubility at their isoelectric point (pH ~4.5). The solubility of soy proteins has been found to increase sharply on either side of the isoelectric point. Most commercial isolated soy proteins range in pH from 4.5 to 7.5; isolated soy proteins with pH near 7.0 have the greatest solubility. Solubility of isolated soy proteins can range from 10% to 90%, with the most functional isolated soy protein having a solubility of greater than 80%. Solubility is related to the gel strength, water holding capacity, emulsification capacity, and foam characteristics. Salt can have a significant negative effect on the solubility of isolated soy proteins (16). The effect of salt can be minimized by proper hydration of the isolated soy protein before the addition of salt (17).

Solubility of isolated soy protein can be controlled through the use of pH, heat, and homogenization during the manufacturing process. The most-soluble commercial isolated soy proteins are produced by using jet cooking, flash cooling, and homogenization at a pH near 7.0. Proteins produced by using optimal processing conditions will have solubility greater than 80% and possess high gelling and viscosity properties. Highly soluble isolated soy proteins are required for maximizing stability of liquid beverage products, emulsification and stabilization of high-fat food systems, textural integrity of meat and dairy analogs, and maximum water binding in meat systems.

Isolated soy proteins with very high solubility are typically not desirable for nutritional bars, powdered beverages, tablet applications, and meat injection or marination systems. In these applications, the solubility of the isolated soy protein is modified to improve the dispersibility of the protein for powdered beverages and injected meat systems and to lower water-binding characteristics for nutritional bar applications.

Gelation

Protein gelation is the result of the formation of partially associated polypeptides, three-dimensional matrices or networks, in which water is entrapped and which exhibit structural rigidity (18,19). Isolated soy protein gels can vary from soft and elastic to hard and brittle in texture. Isolated soy proteins typically do not form gels below 8% concentrations. At concentrations above 10%, isolated soy proteins form soft, nonrigid gels upon heating and cooling. Higher concentrations result in gel formation without heating and these gels become firmer and more elastic upon heating and cooling. Gelation properties of isolated soy proteins are an important consideration in applications where the protein is used to provide a major textural contribution. Meat analogs, dairy analogs such as yogurt, cheese, and sour cream, and highly extended meat products are some of the food systems in which the gelation properties of the isolated soy protein are critical to the structural and textural characteris-

tics of the finished product. Specifics related to the gelling characteristics of isolates for a particular food system are addressed later in this chapter in the food applications section. Gel strength of an isolated soy protein is a function of the processing parameters under which it is manufactured. Factors such as pH, jet-cooking temperature and time, vacuum cooling, spray-drier conditions, enzyme modification, and reducing agent addition can all have a major impact on the gelling properties of the finished product. The use of protease enzymes will typically result in an isolated soy protein that has very low or no gelling properties. Isolated soy proteins that are neutralized to near pH 7.0 and jet cooked at temperatures between 115°C and 150°C will tend to have the highest gelling characteristics (11). Table 7.1 demonstrates the wide range in gelling characteristics that can be achieved through the manipulation of processing parameters.

Emulsification

One of the primary functions of isolated soy proteins is their ability to form stable emulsions in a variety of food systems, including cream soups, meat and meat analog emulsions, dairy analogs, and other high-fat food systems. The definition of an emulsion is a dispersion or suspension of two immiscible liquids (20). Food emulsion systems are much more complex systems that contain both water- and fat-soluble components, such as carbohydrates, proteins, acids, salts, and vitamins. These emulsion systems are further complicated by the processing conditions to which they are exposed, including temperature, pressure, and mechanical agitation. When proteins are used as emulsifiers in a food system, they must be at a concentration sufficient to completely cover the interface of the emulsion, which reduces interfacial tension. The characteristics of proteins that are thought to be most important in emulsification are protein solubility, backbone flexibility, and degree of hydrophobicity (21). Emulsification properties of

TABLE 7.1

Functional Characteristics of Various Isolated Soy Proteins^a

Isolated soy protein	Solubility	pH ^b	Viscosity	Dispersibility	Gelation	Emulsification	Water binding
A	7	7	7	2	7	6	7
B	6	6	1	3	1	7	1
C	4	5	4	5	4	3	4
D	7	7	5	4	5	6	6
E	7	6	3	1	3	7	3
F	2	3	2	6	2	2	2
G	7	6	2	3	2	7	2
H	1	1	1	7	1	1	1

^aRating system: 7 = very high, 4 = moderate, 1 = very low.

^bpH range approximately 7.5 to 4.5, reported as 7 = high (approx. 7.5) and 1 = low (approx. 4.5).

proteins are commonly evaluated by test methods for capacity and stability. In general, emulsion capacity is measured by the continuous addition of oil to a protein slurry; the results are expressed as volume of oil emulsified per unit of protein weight (22). Though this method may work well for evaluating the emulsion capacity of proteins within a given study, comparison of values between studies is difficult because small experimental variations have a significant affect on emulsion capacity results (23).

Isolated soy protein is used as an emulsifier in retorted cream soups, high fat meat and meat analog systems, meal replacement beverages, soy-based mayonnaises, and high-fat dairy analogs. Protein solubility is critical in these applications and isolated soy proteins used in these applications should have a very high degree of solubility. Proper hydration of the isolated soy proteins is essential to ensure maximum emulsification capacity and stability.

Water Binding

Most conventional food systems contain at least 50% water and up to as much as 95% water. Good water binding is essential in these food products. Consumers typically avoid packaged meat products that contain purge (free water) or other food product packages with freestanding water. Formulated food products that have poor water-holding capacity or fat-binding properties have the tendency to lose liquid during the cooking and freezing processes, which results in increased costs of production for the manufacturer. Many other terms have been used to describe water-holding capacity including water binding, hydration capacity, water absorption, water embedding, and water retention (24). Composition and conformational structure of proteins have both been suggested to play a major role in water-holding capacity of a particular protein (25). Water held within a protein structure, such as a gel, is generally categorized into the following two groups: (a) water that is bound to the protein molecule and is not available as a solvent, and (b) trapped water within a protein matrix, which is considered retained water. Bound water is thought to be largely dependent on physicochemical properties including amino acid type, pH, and ionic concentration; retained water is affected more by the structural integrity of the protein matrix such as porosity (26). Most proteins, including isolated soy proteins, bind the least amount of water at their isoelectric point. This is thought to be the result of protonation of the carboxyl groups and enhanced hydrophobic interaction between the protein molecules (27).

The water-holding capacity of isolated soy proteins is critical in many food applications including processed meat, meat analogs, dairy analogs, and bakery applications. Isolated soy proteins with high water-binding characteristics are typically avoided in high protein nutritional bar applications, in which proteins with greater water-binding characteristics cause hardening problems in the bars over extended storage.

Viscosity

Viscosity of a solution is related to the solution's resistance to flow under an applied force. Consumer acceptability of various food systems, such as soups, gravies,

saucers, dressings, and beverages is dependent on the viscosity and consistency of the food product. There are several factors related to proteins that influence the viscosity of a solution or food system, including shape, size, hydrodynamic size (volume or size upon hydration), and flexibility of the protein structure (28). Isolated soy proteins can have a significant influence on the viscosity of food systems. Viscosity of isolated soy proteins can be modified through enzyme modification, the use of reducing agents, or jet cooking and flash cooling conditions. Protease enzyme modification and reducing agents are used to reduce the viscosity of isolates, whereas jet cooking and flash cooling can be used to significantly increase viscosities. Table 7.1 illustrates the wide range of viscosities that can be achieved in these products. Isolated soy proteins that possess high viscosity, solubility, and gel strength are used in products in which viscosity and textural characteristics are important in the food matrix; such foods include meat and meat analogs, dairy analogs, and meal replacement beverages. Isolated soy proteins with lower viscosities are used as emulsifiers in cream soups, high-protein beverages, acidified beverages, infant formula and adult nutrition products, high-protein extruded snacks and cereals, and high-protein nutrition bars. Medium-viscosity isolates are typically the choice in marinated and injected meat systems, meal replacement beverages, and soymilk products.

Dispersibility

There is confusion in the literature and in the protein and food processing industry with regard to definition of dispersibility. Dispersibility has been used to describe and to measure the solubility of soy proteins. The protein dispersibility index (PDI) is an official method of the American Oil Chemists' Society and has traditionally been used to measure the solubility of soy flour products. This terminology has created confusion in the industry that continues even today. The terminology becomes a problem when the terms *dispersibility* and *solubility* are used interchangeably because most highly soluble proteins do not disperse well into aqueous systems. Dispersibility in relation to the incorporation of proteins into a solution or suspension, in general, is defined as the ease with which a protein powder can be dispersed into an aqueous system. The discussions related to dispersibility in this chapter are based on this definition. From Table 7.1, it can easily be seen that dispersibility and solubility are generally inversely related. A highly soluble protein will absorb water quickly at the surface, which causes the protein to form lumps or balls that are dry in the center. Once formed, these lumps or balls are very difficult to break down and eliminate without high shear similar to that achieved through homogenization.

Dispersibility of isolated soy proteins can be modified through changes in pH, lecithination, or agglomeration; each of these either slows or controls the wetting process. Lowering the pH of an isolated soy protein will result in a protein with lower solubility, which slows the wetting process. Lecithin can be applied to the surface of proteins to help control the wetting process. The process of agglomeration produces large porous particles that tend to sink in aqueous systems and are therefore easier to disperse than the smaller spray-dried particles that float on the water and

are difficult to wet. Highly dispersible isolated soy proteins are critical in high-protein powdered beverages in which little or no carbohydrate is added. Isolated soy proteins with good dispersibility are desirable in any application where the protein is to be dispersed into an aqueous system, such as ready-to-drink beverages, dairy analogs, and solutions for injection or marination of whole muscle meats. In these applications, mixers or liquefiers with high shear that do not incorporate large quantities of air are desirable for dispersion of the protein into the aqueous food system.

Foaming and Whipping

The food industry's largest uses of protein-based foams are in the application areas of meringues, mousses, whipped toppings, beer, and a variety of other whipped products (29). Traditional isolated soy proteins have limited foaming or whipping characteristics. Soluble isolated soy proteins exhibit some foaming capacity but virtually no foam stability. The foaming characteristics of isolated soy proteins can be significantly improved by the use of protein fractionation or enzyme modification (30–33). The specialized isolated soy proteins produced through these techniques can possess foaming and whipping characteristics similar to egg white and can be effective in replacing part or all of the egg white in many food applications.

Applications in Food Systems

Isolated soy proteins have been formulated into a large variety of commonly consumed food products. [Table 7.2](#) provides a list of food products in which isolated soy proteins are used and the functional properties the isolates contribute to the food. These proteins can be used simply for protein fortification, for the functional benefits that they bring to a food system, or for the health benefits associated with soy protein. Nutritional bars and beverages are good examples of products in which isolated soy proteins are used to provide the protein nutrient to a food system. In these food systems, isolated soy proteins can also provide some functional benefit. The functional characteristics of isolated soy proteins are discussed earlier in the chapter; some of these characteristics include fat emulsification, structural and textural integrity (e.g., gel strength and viscosity), and water binding. These functional characteristics are discussed in more detail, in relationship to specific food systems, later in this section.

The Food and Drug Administration (FDA) health claim for soy protein that was issued on October 26, 1999 (34), has had a significant impact on the use of soy proteins in food applications. Numerous new food products have been developed in an attempt to take advantage of the high profile of soy foods created in the marketplace as a result of this health claim. In many of these applications, isolated soy proteins are required to achieve the desired soy protein content, given the small reference serving size for some food items. The soy protein health claim allows food manufacturers to make a health claim regarding the heart health benefits of soy protein on their food packaging.

TABLE 7.2**Functional Properties of Isolated Soy Protein in Food Systems**

Food product	Functional properties
Meat products:	
Emulsified: frankfurters, bologna, luncheon meats	Binds water, emulsifies fat, stabilizes emulsion, maintains or enhances texture
Coarse ground: patties, links, sausages, meatballs, pizza toppings	Binds water and fat, improves machinability, enhances texture, improves cooking yield
Injected: ham, roast beef, roast pork, pastrami and other deli meats	Binds water, enhances texture, improves slicing
Marinated: chicken breasts, fajita meats, stew meat	Binds water, enhances eating quality
Surimi	Binds water, whitens product, enhances texture
Vegetarian analogs:	
Coarse ground: burgers, patties, sausage	Binds water and fat, enhances texture, improves product adhesion
Emulsified: franks, luncheon meats, deli loaf	Emulsifies fat, binds water, provides structure/texture
Bakery products:	
White bread	Protein fortification, improves moisture retention
Doughnuts	Improves moisture retention, reduces fat absorption, protein fortification
Cookies and crackers	Protein fortification
Biscuits and muffins	Protein fortification, improve moisture retention
Tortillas	Protein fortification
Nutritional supplements:	
Powdered beverages	Protein fortification, viscosity, mouthfeel
Meal replacement beverage	Protein fortification, fat emulsification, viscosity
Sports nutrition	Protein fortification
Adult nutritional beverages	Protein fortification, fat emulsification and stabilization
Infant formula	Protein fortification, fat emulsification and stabilization
Protein bars	Texture, protein fortification
Protein tablets	Protein fortification
Dairy alternatives:	
Frozen dessert	Fat emulsification, texture
Yogurt	Structure/texture
Milk alternative	Fat emulsification, viscosity
Soft cheese	Structure/texture, fat emulsification and stabilization
Sour cream	Structure/texture, fat emulsification and stabilization
Cheese analogs	Structure/texture, fat emulsification and stabilization
Other foods:	
Soups & sauces	Fat emulsification and stabilization, viscosity
Peanut spreads	Protein fortification, fat binding
Extruded cereals and snacks	Protein fortification
Instant tofu	Structure/texture, fat emulsification and stabilization

The FDA provided the following two model statements, when they issued the health claim for soy protein, that can be used by U.S. food manufacturers on their packaging: “Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease. One serving of (name of food product) provides (quantity of soy protein) grams of soy protein.” Or “25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of (name of food product) supplies (quantity of soy protein) grams of soy protein” (34). For food products to meet the soy protein health claim, a single serving of the food must contain a minimum of 6.25 g of soy protein, be low in fat, saturated fat, and cholesterol, and also meet the general health claim requirements for foods that are the basis of any health claim. Foods made from whole soybeans, such as tofu, may also qualify for the health claim if they contain no fat in addition to that present in the whole soybean. The use of isolated soy protein, to meet the protein requirement for the health claim, is addressed later in this section as each of the specific food systems are discussed.

Before discussions related to the use of isolated soy proteins in specific food systems, the next sections address several general issues regarding the proper use and handling of isolated soy protein. These issues include proper hydration, flavor issues, and proper storage and handling.

Hydration of Isolated Soy Proteins

The functional properties of soluble soy proteins, including isolated soy proteins, are maximized if the protein is properly hydrated during the manufacturing process of a given food. Improper hydration of the protein can result in decreased emulsification capacity and stability, less structural and textural integrity, and insufficient water holding that results in decreased yields upon cooking and freezing, or purge issues during storage. The most important rule in relationship to proper hydration of soy proteins is that the proteins should be hydrated in the absence of salt whenever possible. The solubility and resulting degree of hydration is significantly reduced in ionic environments. This decreased solubility is predominantly determined by the hydrophobic interactions between the proteins and salt. Commercial heat-processed isolated soy proteins have increased hydrophobicity compared to the native soy protein, which results in lower solubility in high ionic environments (17).

Isolated soy proteins for liquid applications such as nutritional beverages, cream soups, and dairy analogs are typically hydrated at 40–50°C for 10–15 minutes before the addition of other ingredients. At lower temperatures, it may be necessary to extend the hydration time. High shear is required to disperse the protein initially, but the agitation should be reduced after dispersion to avoid air entrapment and foam formation. Isolated soy proteins for these applications require a high degree of solubility, similar to isolates in [Table 7.1](#) with solubility values of 6 or 7.

Hydration of the isolated soy protein for emulsified and coarse ground meat systems is usually accomplished through the production of a protein gel. These gels are manufactured in bowl cutters in which one part protein is chopped with four to five

parts water until the protein gel develops a high-sheen appearance, an indication that the protein is sufficiently hydrated. At this point, these protein gels can be incorporated into emulsified and coarse ground meat systems or meat analogs, or can be used to form fat emulsions that can later be used in product manufacture. This is also the process by which the soy proteins are hydrated and fat is incorporated in the production of emulsified meat analogs. These hydration methods continue to be used by meat and meat analog processors throughout the world today. The development of high-throughput operations has resulted in the need for less labor-intense processes for hydration of the protein. This has been accomplished through the development of rapidly-hydrating isolated soy proteins and functional soy protein concentrates. These proteins are added directly to the coarse ground lean meat components in large ribbon or paddle blenders. Addition of the protein to the lean meat results in an increased surface area for protein hydration, which facilitates rapid hydration of the protein upon the addition of the hydration water (five to eight parts per one part protein). This method also works well for coarse ground-style meat analogs in which the textured and powdered dry protein ingredients are incorporated and hydration water added to the mixture during the mixing process before the addition of fat and oil.

Flavor and Odor Issues

In the past, the use of soy proteins in a wide variety of food products has been limited to some extent because of flavor and odor problems. Some of the compounds that have been identified that contribute to the off-flavors associated with soy proteins include carbonyls, alcohols, furans, hydroxy fatty acids, and oxidized lipid fractions (35,36). Many of these compounds also contribute to odor. Boatright and Lei (37) identified several additional compounds in soy that contribute to odors including dimethyl trisulfide, which has been reported to be one of the major contributors to the off-odors of broccoli florets when stored under conditions of reduced oxygen (38). Isolated soy protein products today typically have low flavor and odor profiles. This has been accomplished by the selection of specific soybean varieties that have low flavor and odor profiles, selection of soybeans with low lipoxygenase activity, and control of processing parameters that influence flavor and odor development. Even with continued development in soy protein processing to improve flavor, isolated soy proteins continue to have some degree of off-flavor and odor that may need to be addressed in certain food applications, such as lightly flavored soy beverages and dairy analogs. The flavor industry has recently developed a variety of new masking flavors that are very effective in reducing any residue flavors and odors associated with soy proteins. This has made it much easier for food companies to develop and market a large variety of soy-based foods.

Product Storage and Handling

Most isolated soy proteins are highly functional ingredients. These proteins possess their greatest functional properties on the day of manufacturing and are typically given a shelf life of one year from date of manufacture. Isolated soy proteins are

packaged in materials that provide maximum functionality over time and under good storage conditions (below 25°C and 60% relative humidity). Under conditions of high heat or humidity, the functional characteristics of isolated soy proteins can deteriorate rapidly regardless of the quality of the packaging materials. This decrease in functionality is closely associated with a rapid decrease in protein solubility. As discussed previously, solubility is closely related to the emulsification, gelation, water-binding, and viscosity properties of isolated soy proteins. Food product manufacturers should take storage conditions and time into consideration when using functional soy proteins in their manufacturing facilities. Product developers should make sure that they are working with fresh samples of isolated soy protein and then store these samples in closed containers under the proper storage conditions mentioned above. For best results, the samples can be stored under low humidity, refrigerated conditions, which should significantly extend the shelf life of the isolated soy protein samples.

Health and Nutrition Applications

Nutritional Bars and Other Confectionary-Type Products. The nutritional bar market is the fastest growing segment for soy protein in the health and nutrition area. This nutritional bar arena includes bars targeted for specific demographic and lifestyle groups, including sports nutrition, body building, athletic endurance, women's health, meal replacement, and specialized diet bars (i.e., high protein or low carbohydrate diets). A newly emerging category of nutritional bars includes those that have eating qualities similar to commercially produced confectionary bars (candy bars), but provide some functional health benefit. Chews and other confectionary-type products also fall within this category of health and nutrition products.

Numerous soy proteins are used in nutritional bars: soy flour, soy grits, textured soy flour (TVP[®]), soy protein concentrate (powders, both granular and textured), and isolated soy protein. In most cases, several of the different soy protein products are used to achieve the desired protein content and texture. Soy protein concentrates and isolated soy proteins are being extruded with rice flour, wheat flour, and other ingredients to produce high-protein rice crisps and cookie pieces for use in bars and cereals. These extruded pieces can be used alone or in combination with other soy proteins to produce a finished bar product.

Bar drying and hardening are the most common problems encountered in high-protein nutritional bars. The soy proteins used in these bars can detrimentally affect the drying and hardening properties of bars during storage. This can typically be overcome by the use of isolated soy proteins with the appropriate functional characteristics. Isolated soy proteins with low water-binding characteristics tend to limit the amount of drying and hardening that takes place within the bar during storage. Isolates must provide sufficient textural characteristics to allow the bar to be extruded, but must also have limited water-holding properties to address the drying and hardening issues. Isolated soy proteins similar to C, E, F, and G (Table 7.1) have found use in the nutritional bars in the marketplace today. Highly functional isolates

such as A and D are often used in combination with low water-binding isolates such as B, F, G, and H to produce the desired textural characteristics for manufacturing while minimizing bar drying and hardening during storage and distribution. With regard to the FDA soy protein health claim, the biggest challenge is meeting the low-fat requirement in chocolate-coated bars; otherwise, the 6.25 g of soy protein per serving can be achieved easily in most nutritional bars.

Liquid Nutritional Beverages. There are numerous isolated soy proteins with varying viscosity profiles to help provide the desired consistencies in a variety of liquid beverage products. Isolated soy proteins with very high viscosities can be used to produce milkshake-type products with a thick, rich mouthfeel and texture. Liquid beverages with the consistency of milk require moderate- to low-viscosity isolated soy proteins. Juice-based beverages require isolates with low to very low viscosities so that the protein can be stabilized in the acid environment without producing undesirable viscosity characteristics.

Liquid beverages that incorporate isolated soy protein will be slightly to very cloudy, or opaque, depending on the protein concentration. To date, there are no commercially available isolated soy proteins that will produce a clear liquid beverage. Clear beverages require highly hydrolyzed soy protein products. Even if these were commercially available, there is currently no evidence to show that the heart health benefits would persist in a highly hydrolyzed soy protein product. In general, the protein requirements needed for the FDA soy protein health claim can be easily achieved in most liquid nutritional beverage product applications.

Regardless of the liquid beverage system, it is essential that the isolated soy protein be properly hydrated to obtain the desired results. Highly soluble isolated soy proteins should be hydrated by first slowly adding the protein to water under conditions of high shear; once the protein is dispersed, the agitation should be minimized to avoid air incorporation and limit foam formation. The isolates should then be mixed long enough, typically 10–15 minutes, to ensure proper hydration of the isolated soy protein and maximum functional benefit in the finished product. Insufficient hydration can result in unstable high-fat beverages, beverages with gritty or grainy mouthfeel, or poor product stabilization that requires the use of higher levels of costly stabilizers.

Most liquid beverages that incorporate soy proteins are neutral-based products; however, high-acid and juice-based beverages are also a growing part of the market. All of these products fall within the ready-to-drink (RTD) beverage category. They include beverages for market segments similar to nutritional bars, including sports nutrition, body building, athletic endurance, women's health, meal replacement, drinks for children, specialized diets and adult nutrition products. Shelf-stable products can be produced through ultrahigh-temperature pasteurization (UHT) processing or through retorting. Juice-based, high-acidity products may be thermally processed at lower temperatures and hot-filled into bottles. Liquid beverage products must be formulated for the specific thermal processing conditions that will be used to

manufacture the finished products. Isolated soy proteins require some degree of stabilization regardless of the heat treatment used. Typically, as the severity of the heat treatment increases, so does the stabilization requirement for the beverage system. This is also true for the flavor systems used in these products. Therefore, stabilization and flavor requirements for each beverage system must be developed based on the thermal processing parameters that will be used for manufacture of the particular beverage system. In liquid beverage systems that contain fat, emulsifiers such as mono- and diglycerides are used to help stabilize the fat within the system. Food gum systems are used to provide richness and improve mouthfeel as well as to help stabilize proteins in these liquid systems. Carrageenan, xanthan, locust bean, guar, and cellulose gums are a few of the food gums that can be used to provide these characteristics in neutral-based systems. Pectin alone or in combination with alginate or xanthan is required to stabilize the isolated soy proteins in high-acidity beverages.

Many liquid beverage products are calcium-fortified to provide calcium levels similar to those found in milk and other dairy products, since isolated soy proteins typically have low calcium content. Soy proteins are very sensitive to calcium ions and will coagulate or aggregate when exposed to highly ionized, soluble calcium salts (e.g., calcium chloride or dairy calcium sources). Insoluble calcium sources such as tricalcium phosphates cause limited, if any, aggregation of soy protein. Micronized tricalcium phosphate is the preferred calcium source for these applications as well as dairy analog applications because it is easily suspended in liquid beverage systems by the stabilization systems normally used in these products. Sequestering agents are commonly used to interact with any free divalent ions that might cause aggregation of the isolated soy protein in liquid beverage systems. These sequestering agents include polyphosphate compounds such as sodium or potassium hexametaphosphate and sodium or potassium citrates. These compounds can be used alone or in combination to help protect the stability of the isolated soy protein.

Each beverage application requires the selection of an isolated soy protein that possesses the functional characteristics needed for the particular application. Isolated soy proteins produced for powdered beverage applications are seldom appropriate for liquid beverage applications and vice versa. Regardless of the liquid beverage application, the isolate should be bland in flavor and have a high degree of solubility. Soluble proteins are critical to maintain protein stability within the liquid beverage system. Isolates similar to A, D, and E ([Table 7.1](#)) can be used in most neutral-based liquid beverage systems including meal replacement products, sports drinks, women's health, and flavored drinks for kids. High-protein drinks used for muscle building or low carbohydrate diets require lower-viscosity isolates such as B and G. These products are required to maintain desirable viscosity characteristics in the finished products. High-acidity and juice-based liquid beverages require isolates with viscosity characteristics similar to those for high protein beverages (i.e., B and G). As explained previously, these are necessary to maintain a low viscosity profile in the high-acidity and juice-based beverage while providing the required stabilization for the protein.

Homogenization is an important processing requirement in the production of quality liquid beverages. Homogenization helps break down protein particles and improves the mouthfeel and textural characteristics as well as ensuring proper emulsification of added fat. Two-stage homogenization is preferred and produces the best results in soy-based liquid beverages. Homogenization pressures of at least 2500/500 psi are desirable at temperatures between 70°C and 90°C. In high-acidity (low pH) beverages, homogenization is a critical part of the process in that it further activates the pectin and improves stabilization of the protein.

Powdered Nutritional Beverages. Dry powdered beverages require isolated soy proteins with different functional characteristics than isolates for liquid beverage applications. The most important functional and physical characteristics in powdered beverages are dispersibility and density. Density is important in two areas. First, higher-density isolates have advantages in packaging and shipping, since larger quantities (by weight) can be put into a smaller space; and second, higher-density products tend to have better flow characteristics. As discussed previously, dispersibility relates to the ease with which a protein powder can be dispersed into an aqueous system. The more dispersible a protein product, the less shear is required to disperse the product in an aqueous system.

The powdered beverage industry continues to search for ways to improve the dispersibility of their products to meet the consumers' demand for products that can be put into solution either by the use of a shaker cup or by simply stirring the product into solution with a spoon. There are several methods that are used to improve the dispersibility of isolated soy proteins. The first involves lowering the pH of the isolate, which in turn lowers the solubility of the protein and also can increase density. However, as you move further away from neutral pH and closer toward the isoelectric point of the protein, isolated soy protein begins to contribute more of a gritty or grainy texture and mouthfeel in the powdered beverage product. Food gums such as xanthan, locust bean, cellulose, and carrageenan can be used to provide a smoother mouthfeel to these dry powdered products. Isolated soy proteins C and F (Table 7.1) are two proteins that have lower pH and improved dispersibility. Isolate C would be a better choice for powdered beverages because it has slightly higher solubility, has moderate dispersibility, and should contribute less grittiness and graininess to finished powdered beverages. Lecithination can be used to improve dispersibility of isolated soy proteins through controlling the wetting process; however, even with the addition of lecithin to the surface of highly soluble isolated soy proteins there is a tendency for the protein to form clumps or lumps upon dispersion into a liquid system under low shear (e.g., isolates B, D, and G, Table 7.1).

The most dispersible, highly-soluble isolated soy proteins are those that are agglomerated. The agglomeration process produces large porous particles that tend to sink in aqueous systems and are therefore easier to disperse than the smaller spray-dried particles that float on the water surface and are difficult to wet out. These highly dispersible isolated soy proteins produce the best results in high-protein powdered beverages where little or no carbohydrate is added. When agglomerated isolated soy

proteins that are highly dispersible and soluble are used in the manufacture of dry powdered beverage products, the finished products disperse easily in liquid systems, stay in suspension (no settling), and have a smooth texture and mouthfeel. Isolates B, D, E, and G (Table 7.1) are good potential proteins for agglomeration.

Viscosity of the dry powdered beverages can be modified to some extent by the isolated soy protein that is selected. For example, isolates B, D, and G have similar solubility and dispersibility characteristics, but range from moderate to very low viscosity. If a high-viscosity beverage is desired, isolate A would contribute the most to the viscosity of the finished beverage. Food gums and cellulose gels can be added if additional viscosity is required.

Protein Tablets. Isolated soy proteins used to produce protein tablets for nutritional supplements such as isolates F and H in Table 7.1 are typically of very high density and have low solubility. These characteristics are required in the isolated soy protein to achieve the desired degree of compaction necessary for production of stable tablets.

Clinical and Pediatric Nutritional Products

Isolated soy proteins for these markets require high-quality proteins that can support the nutritional requirements of growing children as well as providing nutritional protein requirements for tube-fed and oral nutritional supplements. These products are typically specialty isolated soy protein products, some of which are fortified with calcium in order to provide calcium-to-phosphorus ratios equivalent to milk. Many of the isolates for these applications have functional characteristics similar to those for liquid nutritional beverage products that have already been discussed. Isolated soy proteins for these applications must have a high degree of solubility and excellent emulsification properties because fat is a major nutrient requirement in the finished products. These isolates are also available with a range of viscosity profiles (very low to moderately high) to meet the needs of the specific nutritional products. Some of the finished products in which isolates are used include liquid (RTD), concentrate, and powdered infant formula products, cereals for weaning, and a variety of other food products developed for toddlers.

Isolated soy proteins are used in these applications to provide alternatives to milk for infants and toddlers with milk-intolerance problems. Isolated soy proteins are used in tube-fed and oral supplements as an economical protein source that possesses the nutrient quality and product functionality required for the particular application. Specialty isolates have been developed for tube-fed and oral supplements that can meet the desired viscosity and flow characteristics required in the products.

Meat Product Applications

Isolated soy proteins are used in a variety of processed meat applications including injected and marinated, coarse ground, emulsified, and dry fermented meats to bind water, emulsify fat, and provide structural and textural integrity. Specific functional

requirements for the isolates differ for each processed meat application. The use of isolates as well as other soy proteins in meat applications is regulated in most countries throughout the world, and these regulations differ from country to country. The specific regulations for each country should be consulted before the use of soy protein in any processed meat product application. Specialty low-nitrite and -nitrate isolated soy proteins are produced for use in uncured red meat and poultry applications. These products are produced under specific processing conditions to ensure that very low nitrite and nitrate levels are achieved in the isolates to avoid the occurrence of cure meat reactions in uncured meat applications such as roast beef, chicken and turkey breast, beef patties, chicken patties and nuggets, pizza topping, meatballs, and meatloaf.

Injection and Marination Applications. Hams, roast beef, pastrami, corned beef, roast pork, fish fillets, turkey breasts, and other whole muscle deli meats are a few of the meat products that can be produced through the use of injection technologies. Isolated soy protein can be combined with salt, phosphate, sugars, starches, and food gums (e.g., carrageenan) to produce an injectable brine solution. This solution is injected into intact muscles pieces, and injected muscles are tumbled or massaged to distribute the solution and extract salt-soluble muscle proteins, and then either cooked or frozen. Isolated soy proteins can improve the slicing properties, reduce purge, enhance firmness, and reduce shrinkage of injected meat products. Whole muscle marination is accomplished in a similar manner, but the products are tumbled with the marinade rather than being injected. Marination can be used to enhance the eating quality (e.g., succulence) as well as holding properties of processed meats in high-abuse circumstances such as products that are held for extended periods on steam tables. Whole muscle meats such as chicken breasts, chops, steaks, shrimp, stew meats, and fajita meat pieces are a few of the meats in which marinades are used. Isolated soy proteins are also used to bind moisture and provide textural characteristics in marination applications. Isolated soy proteins used in injection and marination applications have characteristics similar to isolates A, C, and D in [Table 7.1](#). Proper hydration of these proteins in the absence of salt is critical to achieve the desired functional water holding properties and structural integrity of these proteins.

Coarse Ground Meats. Isolated soy proteins are used to provide texture and cohesiveness, absorb fat, and bind water in coarse ground meat systems. Isolates can be added dry to the product during processing or can be manufactured into a gel-like material that simulates ground meat prior to addition to the meat system. Highly functional isolates, such as A and D ([Table 7.1](#)), can be used in coarse ground meats such as patties, nuggets, meatballs, meatloaf, pizza toppings, sausages, and restructured fish products (cakes and sticks).

Emulsified Meats. Emulsified meat products have traditionally been the largest application for isolated soy proteins in processed meats. Functionally, isolates provide effective fat emulsification, structural and textural integrity, and water binding.

Isolated soy proteins can also reduce purge and improve product yield. Isolates used in emulsified meat applications have good emulsification properties, are highly soluble, and have moderate to high gelling characteristics. This would include isolates similar to A and D in [Table 7.1](#). In emulsified meat applications in which lean meat content is limited and the isolated soy protein is used at levels of greater than 3%, isolates with the highest gelling characteristics are required to maintain textural integrity. The production of gels and emulsions has been used commonly in emulsified meat to ensure that the protein is fully hydrated and that the maximum functional benefit can be achieved. As discussed previously, dry addition is gaining popularity worldwide as more continuous, lower cost (labor) systems are being used for product manufacture.

Dry Fermented Meats. Dry fermented meats include products such as salami and pepperoni. Isolated soy protein can be used to replace lean muscle protein for cost-reduction measures or be used to replace fat for the production of reduced-fat products. This can be accomplished through the production of protein gels that have been reduced in particle size to simulate ground lean meat or fat. Isolated soy proteins used in this application require very high gelling properties. Materials produced for the replacement of lean meat are usually colored to produce protein particles that resemble the color characteristics of the cured red meat being replaced. One of the major benefits of this process is that meat-like texture and good particle definition can be maintained in reduced-fat products as well as in products with reduced lean meat content. Isolates can also be added in the dry form to the fermented meat product during the manufacturing process. Addition of the isolate increases protein content and decreases the moisture-to-protein ratio, which can shorten drying time and increase product throughput. Isolates used for dry addition to dry fermented sausages usually have functional characteristics and pH in the moderate-to-low range, where high gel strength, emulsification, water binding, and solubility are not typically desired. Isolates with these functional characteristics tend to allow for quicker drying under traditional drying conditions.

Meat Analogs Products

There are several forms of soy proteins that are used in meat alternative products. Vegetarian patties and sausages can contain textured soy flour (e.g., TVP®) and textured soy protein concentrates as well as functional soy proteins such as soy protein concentrates and isolated soy proteins. There are four types of meat analogs: fine emulsions (franks, hotdogs, and bologna types), coarse ground-type products (patties, links, and nuggets), crumble, strip, or chunk types (ground beef, chicken, or beef-type strips), and emulsions with particulates (chicken, bacon, luncheon meat and ham type products). Fine emulsions are products that typically use isolated soy protein alone or in combination with functional soy protein concentrates. These functional soy proteins provide both textural and emulsification properties. In a vegetarian frankfurter, the isolated soy protein provides much of the structural and textural characteristics of the product as well as functions to bind any fat in the system. Coarse ground systems are products made

with combinations of textured soy proteins (TVP® and textured soy protein concentrates) and functional proteins (isolated soy proteins and soy protein concentrates). The textured products provide coarse ground meat-like texture, while the functional proteins help bind the product together and help with moisture and fat retention. Crumble, strip, and chunk products have some similarities to coarse ground meat analog products, except that these products simulate meat products such as strips and chunks of meat or browned ground beef and sausage-type products. Textured soy proteins (TVP® and textured soy protein concentrates) are hydrated with meat-type and other flavoring and seasoning systems to produce the finished textured pieces. These hydrated pieces can be individually quick frozen (IQF) and sold as an ingredient for cooking, or incorporated into complete meal entrees. Emulsions with particulates are products that use a combination of textured and functional soy proteins in which the major component of the product is present in the emulsion phase.

The major challenge in the development of meat alternative products is the achievement of textural and flavor properties similar to the comparable meat product that the analog is intended to replace. The flavors for these meat analog products must be made from nonmeat materials, yet possess the flavor characteristics of meat. Reaction flavor technology has allowed for the development of these types of flavors. This technology uses processes that react naturally-occurring reducing sugars with amino acids, amines, peptides, and proteins in order to produce complex flavor compounds, many with the natural flavor characteristics associated with meat.

The textural characteristics of meat analogs continue to pose a challenge for product formulators; however, new technologies are emerging for producing the textural characteristics in meat alternative products that more closely simulate the texture of meat. Isolated soy proteins have a major role in providing structural and textural characteristics to many of these meat analog products. The isolates that are used in these applications must possess high gelling and emulsification properties, such as isolates A and D in [Table 7.1](#). Isolated soy proteins are used at high concentrations in meat alternative frankfurters, deli loafs and slices, and, to a lesser extent, in patties and links. In meat alternative products (other than crumbles, strips, and chunks) additional functional ingredients are used to further enhance the textural characteristics of these products. Current technologies employ the use of egg albumen, vital wheat gluten, cellulose gums, modified starches, protein cross-linking enzymes (i.e., transglutaminase), and other specialty food gums for the development of the desired textural characteristics in these products.

Many of the meat analog products on the market in the United States today meet the FDA soy protein health claim requirements. The difficulties that are encountered in producing products to meet the health claim are related to reaching the low fat and sodium requirement while maintaining overall product quality in the areas of juiciness and flavor.

Extruded Cereals and Snacks

Isolated soy proteins can be used in extruded cereals and snacks to significantly increase the protein content of these products. In developing countries, isolates are

used, in many cases, simply for protein fortification. In the United States, isolated soy proteins are used in extruded snacks and cereals to produce products to meet the needs of the high-protein diet market for low-carbohydrate traditional foods or to meet the FDA soy protein health claim. Isolated soy proteins and soy protein concentrates have been successfully extruded with rice flour and other ingredients to produce high protein rice crisps, oat rings, cookie pieces, chips, and curls. Many of these extruded rice crisps and cookie pieces are used in the manufacture of nutritional bars. Isolates for these extrusion applications need to possess low water-binding characteristics that allow for the proper puffing or sheeting of the products during manufacture. These proteins are typically low in viscosity and possess little if any gelation properties. Isolated soy proteins with functional characteristics similar to isolates B, E, and G (Table 7.1) tend to work the best in these extrusion applications.

Bread and Other Baked Goods

Breads, rolls, buns, bagels, pretzels, cakes, muffins, crackers, and tortilla products are only a few of the types of baked goods for which new products are being developed to address the FDA soy protein health claim. Isolated soy proteins have not traditionally been used as ingredients in these products; however, there has been considerable interest from the bakery industry with regard to the incorporation of soy proteins into baked goods ever since approval of the FDA health claim. In products such as cookies, crackers, and muffins, it can be difficult to achieve the level of soy protein required to meet the soy protein health claim even with isolated soy proteins. This is, in part, because of the small reference-serving size for these particular foods; however, isolated soy proteins provide the greatest opportunity to achieve the highest possible protein content in such products. In other bakery products, it is an easier task to develop products to meet the health claim. In products such as breads and bagels, it may be necessary to adjust the ratio and levels of dough conditioners, enzymes, and leavening agents to achieve the desired results. It is important in these bakery products to use soy proteins that have as little effect as possible on the physical properties of the baked goods. The isolated soy proteins that are used in these bakery applications must have very low water-binding characteristics, such as the isolates found in Table 7.1 with water-binding values of 3 or below. Isolated soy proteins can be used in traditional bakery products for moisture retention and to reduce fat absorption in fried bakery products such as doughnuts. In these products, isolated soy proteins with high water-binding characteristics will achieve the desired results at the lowest cost. Isolated soy protein can also be used to improve the glaze and gloss retention of baked goods.

Dairy Alternative Products

In the production of dairy alternative products, there are several issues and characteristics that are similar with regard to product development. In each case, the iso-

lated soy proteins are used as the functional protein source. Isolates for these applications must be clean flavored, light in color, highly soluble, and have good emulsification properties. The major objective in the development of dairy alternative products for the United States and other industrialized countries is high-quality products with eating characteristics that are similar to their dairy counterparts. In underdeveloped countries the objective is typically the production of the most economical products possible that meet the desired nutritional requirements with acceptable sensory characteristics.

Production of dairy alternative products that have eating qualities similar to dairy products requires the incorporation of flavor masking and dairy-type flavors. Flavor masking technology is used to help minimize any undesirable flavor notes that may be associated with the isolated soy protein used. Less masking should be required in the future as isolated soy protein manufacturers continue to improve flavor through selection of higher-quality raw materials as well as improving the manufacturing process.

Each dairy product has unique flavor characteristics that may be associated with the beginning raw material (i.e., milk), the manufacturing procedures (e.g., aging of cheese), or the fermentation processes (e.g., culturing of yogurt). Product formulators must incorporate these unique dairy flavor notes into analog products through the use of dairy-derived flavors (containing dairy components) or dairy-type flavors (dairy-free). If the dairy alternative product is to be marketed as a dairy-free product, then dairy-type flavors should be used in product development.

Soymilks. Soymilks have traditionally been manufactured through the use of whole-bean processes in which the soybeans are soaked in water, washed, and ground. This ground material is then filtered through cloth and the filtrate is heated to produce the final soymilk product. This process has been modified and improved over the years to produce lightly flavored products that continue to gain greater acceptance throughout the world. Isolated soy protein can be used in combination with fat and carbohydrate sources as well as with stabilizer systems in order to produce comparable products. A calcium source is typically formulated into the product to ensure that its calcium level is similar to that of milk. Vitamins A and D are also formulated into soymilk products in many cases. The advantage to the use of isolated soy proteins for the manufacture of soymilk products is that the soymilk can be produced on equipment commonly used in dairy processing plants. This allows established dairy processing plants to begin producing soymilk in their existing facilities with little or no additional capital expenditures. As in any liquid beverage system, isolated soy proteins that are bland in flavor, light in color, and have high solubility are the isolates of choice. Those isolates with the appropriate solubility and moderate-to-high viscosity properties are typically used in soymilk applications (A and D, [Table 7.1](#)). Soymilks made with isolated soy protein can easily be formulated to meet the FDA soy protein health claim.

Yogurts. Isolated soy proteins in combination with fat and carbohydrate source can be used to formulate nondairy yogurt products with similar nutrient content to dairy yogurts. As with soymilk, calcium and vitamins A and D can also be added. The functional characteristics of isolates for soy yogurt include high solubility, moderate-to-high viscosity, good emulsification, and water binding. Isolates similar to A and D (Table 7.1) provide these desired characteristics. The isolated soy proteins are generally responsible for the structural and textural characteristics in these yogurt products. Yogurt products manufactured with isolated soy proteins require stabilization similar to their dairy counterparts. These soy yogurts are also fermented products and require the use of cultures similar to those used in the production of dairy yogurt. In general, soy yogurts require longer fermentation time than dairy yogurts. Many of the same fruit preparations that are used in dairy yogurts can also be used in soy yogurts. Flavor masking in combination with dairy-type flavors are necessary in order to develop the desired flavor characteristics in the finished yogurt products.

Soy yogurts can easily be formulated to meet the FDA soy health claim requirements. Isolated soy proteins can also be used in conjunction with milk to produce dairy yogurts that contain the 6.25 g of soy protein required to meet the soy protein health claim. These products possess the traditional characteristics of dairy yogurt and require little modification to the traditional processes for making dairy yogurts.

Sour Creams and Soft Cheeses. Isolated soy proteins are used for their emulsification properties and their contribution to the structure and texture of nondairy sour cream and soft cheese products. These sour cream and soft cheese products are formulated with combinations of fat, carbohydrate, stabilizers, and flavors. Once the product bases are put together, the processing parameters are similar to the comparable dairy products. In addition to emulsification properties, isolates for these applications must have high solubility. Isolates A, D, and E from Table 7.1 have functional characteristics similar to those needed for nondairy sour cream and soft cheese applications.

Frozen Desserts. Frozen desserts manufactured with isolated soy proteins are formulated and processed in a way similar to their dairy counterparts. Isolated soy proteins are used as the protein source in these products. The proteins must be highly soluble, very clean in flavor, and have excellent emulsification properties with moderate-to-high viscosity characteristics. Isolated soy proteins similar to A and D (Table 7.1) would have the functional characteristics desirable for frozen dessert applications. As with soy yogurts, many of the fruit and flavor preparations used in the manufacture of ice cream also work well in a frozen dessert application made with isolated soy protein.

Other Processed Foods

Pasta. Various protein sources, including isolated soy protein, have been investigated for use in protein fortification of pasta to improve the nutritive value. Sosis and

Young (39) showed that isolated soy protein could be added to either hard or soft wheat or to blends with durum semolina in order to provide comparable physical characteristics to products manufactured with pure durum semolina. This study showed that lower-cost wheat could be used in combination with isolated soy protein to produce acceptable pasta products and provide an advantageous contribution to protein content and quality in the pasta. Through the addition of isolated soy protein, high-protein pasta products have been developed that meet the FDA requirements for the soy protein health claim and requirements for school lunch programs in the United States. These high-protein products can also provide alternative choices for individuals who are trying to limit the amount of carbohydrate in their diets. The isolates that are used in these pasta application have moderately to highly functional characteristics (i.e., isolates A, C, and D, [Table 7.1](#)); however, processing conditions (i.e., mixing and extruding) within a given manufacturing facility play a major role in determination of the appropriate isolated soy protein.

Soups and Sauce. Isolated soy proteins can be used in soups and sauces for protein fortification but are more traditionally used for the functional benefits. In retort canned cream soup applications, isolates serve the function of emulsifying fat and stabilizing the emulsion during the retort process. These proteins can also help increase product viscosity and provide mouthfeel and texture. Similar functional benefits can be achieved in other soup and sauce applications through the use of functional soy protein concentrates. Isolates for these applications must have high solubility and excellent emulsification properties with moderate to low viscosity similar to isolates D, E, and G ([Table 7.1](#)).

Reduced-Fat and Other Spreads. Reduced-fat peanut spreads lead this category of products, but the category also includes products such as soy mayonnaise, salad dressings, and soynut butters. Isolated soy proteins are used in reduced-fat peanut spreads to maintain protein content (protein fortification) and fat absorption. The isolates that have been used in this application have functional properties similar to isolates C, D, and E ([Table 7.1](#)). Isolated soy proteins for soy mayonnaise and salad dressing are typically those that have high solubility and low viscosity, such as isolates B and G. Soynut butters are normally manufactured from roasted soybeans, but can also include isolates for the purpose of fat binding and protein fortification.

Summary

Isolated soy protein technology has continued to evolve over the past 60 years. Through technological development, the isolates being produced today are bland in flavor, light in color, and possess a wide variety of functional characteristics. These functional characteristics include gelation, viscosity, emulsification, water binding, and, to a limited extent, foaming and whipping. It is essential that these isolated soy proteins have a high degree of solubility to achieve the maximum functional properties.

These soluble proteins must also be properly hydrated to take full advantage of their functional characteristics.

Isolated soy proteins are incorporated into food systems for a variety of purposes. These proteins may be used in food systems simply for protein fortification, for the functional properties they impart, or for the health benefits associated with the consumption of soy protein. Isolated soy proteins can be used in nutritional bars and beverages, baked goods, processed meats, meat and dairy alternatives, clinical and pediatric nutrition, cereals and snacks, soups and sauces, and reduced-fat spreads and pasta, to mention a few. Regardless of their intended use, selection of the appropriate isolate soy protein is critical for successful product development. If care is used in the isolated soy protein selection process, many of the frustrations associated with product development can be averted. The soy protein manufacturing technical staffs are the best sources of information with regard to selection of the proper soy protein for product development. Product developers should remember to store these isolated soy proteins in a cool, dry environment and to make sure that they are working with protein samples that are no more than 6–8 months old.

Soy protein technology will continue to improve in the years to come, with further improvements in the areas of flavor, color, and functional and nutritional properties. As researchers learn more about the health benefits related to the consumption of soy, mainstream consumer demands for a wider variety of soy-containing foods will continue to increase. Mainstream consumers will expect these soy-containing foods to be good-tasting and of the highest quality. Through continued consumer education with regard to the health benefits of soy and the development of superior quality soy foods, the future for soy protein appears very positive.

References

1. Cone, C.N., and E.D. Brown, Protein Product and Process of Making, U.S. Patent 1,955,375, April 17, 1934.
2. Julian, P.L., and A.G. Engstrom, Process for Production of a Derived Vegetable Protein, U.S. Patent 2,238,329, April 15, 1941.
3. Erkkö, E.O., and R.T. Trelfa, Process for the Isolation of Soybean Protein, U.S. Patent 2,460,627, February 1, 1949.
4. Eberl, J.J., and R.T. Trelfa, Process for Isolating Undenatured Soybean Protein, U.S. Patent 2,479,481, August 16, 1949.
5. Turner, J.R., Modified Soy Protein and the Preparation Thereof, U.S. Patent 2,489,208, November 22, 1949.
6. Sair, L., and R. Rathman, Preparation of Modified Soy Protein, U.S. Patent 2,502,029, March 28, 1950.
7. Sair, L., and R. Rathman, Preparation of Modified Soy Protein, U.S. Patent 2,502,482, April 4, 1950.
8. Circle, S.J., P.L. Julian, and R.W. Whitney, Process for Isolating Soya Protein, U.S. Patent 2,881,159, April 7, 1959.
9. Anson, M.L., and M. Pader, Extraction of Soy Protein, U.S. Patent 2,785,155, March 12, 1957.

10. Sair, L., Method of Extracting Protein from Defatted Soybean Material, U.S. Patent 3,001,875, September 26, 1961.
11. Hawley, R.L., C.W. Frederiksen, and R.A. Hoer, Method of Treating Vegetable Protein, U.S. Patent 3,642,490, February 15, 1972.
12. Frazeur, D.R., and R.B. Huston, Protein and Method of Extracting Same from Soybeans Employing Reverse Osmosis, U.S. Patent 3,728,327, April 17, 1973.
13. Gomi, T., Y. Hisa, and T. Soeda, Process for Preparing Improved Soy Protein Materials, U.S. Patent 4,113,716, September 12, 1978.
14. Gomi, T., Y. Hisa, and T. Soeda, Process for Preparing Improved Soy Protein Materials, U.S. Patent 4,186,218, January 29, 1980.
15. Walsh, J.E., Process for the Production of a Protein Isolate Having Improved Whiteness, U.S. Patent 4,309,344, January 5, 1982.
16. Shen, J.L., Solubility and Viscosity, in *Protein Functionality in Foods*, edited by J.P. Cherry, ACS Symposium Series 147, American Chemical Society, Washington, D.C., 1981, pp. 89–109.
17. Furukawa, T., and S. Ohta, Solubility of Isolated Soy Protein in Ionic Environments and an Approach to Improve its Profile, *Agric. Biol. Chem.* 47:751–755 (1983).
18. Catsimpoilas, N., and E.W. Meyer, Gelation Phenomena of Soybean Globulins. I. Protein-Protein Interactions, *J. Am. Oil Chem. Soc.* 47:559–570 (1970).
19. Kinsella, J.E., Functional Properties of Soy Proteins, *J. Am. Oil Chem. Soc.* 56:242–258 (1979).
20. Dickinson, E., and G. Stainsby, *Colloids in Foods*, Applied Science Publishers, London, 1982.
21. Hill, S.E., Emulsions, in *Methods of Testing Protein Functionality*, edited by G.M. Hall, Blackie Academic & Professional, an imprint of Chapman & Hall, London, 1996, pp. 153–185.
22. Swift, C.E., C. Lockett, and P.J. Fryer, Commintuted Meat Emulsions—The Capacity of Meat for Emulsifying Fat, *Food Technol.* 15:469 (1961).
23. Sherman, P., A Critique of Some Methods Proposed for Evaluating the Emulsifying Capacity and Emulsion Stabilizing Performance of Vegetable Proteins, *Ital. J. Food Sci.* 1:3–4 (1995).
24. Kneifel, W., and A. Seiler, Water Holding Properties of Milk Protein Products—A Review, *Food Struct.* 12:297–308 (1993).
25. Kinsella, J.E., D.M. Whitehead, J. Brady, and N.A. Bringe, Milk Proteins: Possible Relationships of Structure and Function, in *Developments in Dairy Chemistry—4. Functional Milk Proteins*, edited by P.F. Fox, Elsevier Applied Science, London, 1989, pp. 55–95.
26. Kneifel, W., P. Paquin, T. Abert, and J.P. Richard, Water-Holding Capacity of Proteins with Special Regard to Milk Proteins and Methodological Aspects—A Review, *J. Dairy Sci.* 74:2027–2041 (1991).
27. Knutz, I.D., Hydration of Macromolecules: III. Hydration of Polypeptides, *J. Am. Chem. Soc.* 93:514–516 (1971).
28. Damodaran, S., Amino Acids, Peptides and Proteins, in *Food Chemistry*, 3rd ed., edited by O.R. Fennema, Marcel Dekker, Inc., New York, 1996, pp. 322–429.
29. Wilde, P.J., and D.C. Clark, Foam Formation and Stability, in *Methods of Testing Protein Functionality*, edited by G.M. Hall, Blackie Academic & Professional, an imprint of Chapman & Hall, London, 1996, pp. 153–185.

30. Turner, J.R., Modified Soy Protein and the Preparation Thereof, U.S. Patent 2,489,208, November 22, 1949.
31. Gunther, R.C., Vegetable Aerating Proteins, U.S. Patent 3,814,816, June 4, 1974.
32. Davidson, R.M., R.E. Sand, and R.E. Johnson, Method for Processing Soy Protein and Composition of Matter, U.S. Patent 4,172,828, October 30, 1979.
33. Lehnhardt, W.F., and F.T. Orthoefer, Heat-Gelling and Foam-Stabilizing Enzymatically Modified Vegetable Isolates, U.S. Patent 4,409,248, October 11, 1983.
34. Food and Drug Administration, Food labeling: Health Claims: Soy Protein and Coronary Heart Disease, *Fed. Reg.* 64:206 (Oct. 26, 1999).
35. Wolf, W.J., Lipxygenase and Flavor of Soybean Protein Products, *J. Agric. Food Chem.* 23:136–141 (1975).
36. Sessa, D.J., and J.J. Rackis, Lipid-Derived Flavors of Legume Protein Products, *J. Am. Oil Chem. Soc.* 54:468–473 (1977).
37. Boatright, W.L., and Q. Lei, Compounds Contributing to the “Beany” Odor of Aqueous Solutions of Soy Protein Isolates, *J. Food Sci.* 64:667–670 (1999).
38. Hansen, M, R.G. Buttery, D.J. Stern, M.I. Cantwell, and L.C. Lang, Broccoli Storage under Low-Oxygen Atmosphere: Identification of Higher Boiling Point Volatiles, *J. Agric. Food Chem.* 40:850–852 (1992).
39. Sipos, E.F., and L.L. Young, Pasta Product, U.S. Patent 4,000,330, December 28, 1976.

Chapter 8

Barriers to Soy Protein Applications in Food Products

Leslie Skarra

Merlin Development, Plymouth, MN 55441

Soy protein applications have historically focused on use of unique functional properties offered by soy or replacement of more expensive ingredients. The Food and Drug Administration (FDA) health claim for soy protein and the increasing popularity of “low-carb” products provide major new opportunities for soy applications by driving broader range mainstream consumer products that contain a high level of soy protein. However, the taste and functionality of soy ingredients continue to present significant barriers to successful product development. Traditional soy applications require maximum functionality to permit low levels, which minimizes both cost and effect on the food system. However, delivery of high levels of soy protein required to meet the health claim and low-carb requirements drive a totally new set of considerations.

As previous experience with reduced fat products shows, the window of opportunity to deliver great tasting products is limited. Therefore, the need for improvement and alternatives is urgent. In the previous three chapters, three major soy protein products—flour, concentrate, and isolate—are discussed in detail with respect to production technology, product properties, and applications, respectively. In this chapter, specific concerns for product development with soy protein products and possible solutions are discussed. In addition, as more manufacturers use soy in a wider variety of applications, other manufacturing trends will drive relevant considerations.

Historical Focus of Soy Protein Market

Although soybeans have been part of the Oriental diet for thousands of years, they are a relative newcomer to the United States, first introduced at the beginning of the twentieth century. Initially valued as a source of oil, the protein by-product was relegated to animal feed. However, persistent technology developments have expanded forms, uses, and economic value of soy protein (1).

Initial applications of soy flour in bakery products exploited soy’s unique functional ability to improve bread dough mixing, baked crumb color, moisture holding, and shelf life properties. Use levels were as low as needed to achieve the desired end effect. This served to maximize the economics of soy application and to minimize the impact of any off-flavors or negative textural contributions in the finished product.

As isolates were developed in the 1950s and concentrates in the 1960s, the functional properties of soy protein were clarified and applications expanded. The general thrust of these applications was twofold, as follows: (a) applications that used a unique property of soy protein, and (b) applications that replaced a more expensive ingredient with soy protein, resulting in a cost savings while preserving the process characteristics, taste, texture, and keeping qualities of the finished product.

These applications focused on using the lowest level of soy protein possible to achieve the intended effect. If any negative attributes of soy were evident in the finished product, the use level of soy could be reduced to eliminate the negative effects. Meanwhile soy protein manufacturers focused significant effort on (a) developing new soy protein products with additional desirable properties, (b) minimizing negative attributes of soy protein products, and (c) minimizing costs for soy protein applications.

U.S. consumption of soy protein products gradually increased via inclusion of soy protein ingredients in mainstream consumer products. As shown in Table 8.1,

TABLE 8.1

Some Products Containing Soy Protein^a

Bakery Products

Bread, rolls
Specialty breads
Cakes, cake mixes
Cookies, biscuits, crackers
Pancakes, sweet rolls
Doughnuts

Dairy-Type Products

Beverage powders
Cheeses
Coffee whiteners
Frozen desserts
Whipped toppings
Infant formulas
Milk products
Milk replacers for young animals

Miscellaneous Applications

Candies, confection, desserts
Dietary items
Asian foods
Pet foods
Soup mixes, gravies

Meat Food Products

Emulsified meat products
Bologna, frankfurters
Miscellaneous sausage
Luncheon loaves
Canned luncheon loaves
Seafoods

Ground meat products

Chili con carne, sloppy joes
Meat balls
Patties
Pizza toppings
School lunch/military
Seafood

Whole-muscle meat

Analogs
Ham
Meat bits (dried)
Poultry breast
Seafood (surimi)
Stews

^aData from Endres (31).

soy protein applications have clearly focused on products that benefit from functional properties of soy or cost savings and yield improvements.

Soy for Health Uses

Soy for Vegetarians

Soy, with its high quality protein, is an ideal vegetarian food. The research to enhance soy protein's ability to cost effectively replace meat protein also provided a variety of ever-improving products to service the vegetarian market. Vegetarian products were marketed through a relatively separate system of health food stores and natural markets until relatively late in the 1990s, when they began a significant migration into traditional grocery stores. This migration was driven, in part, by disease concerns in meat and perceived opportunities for health enhancement offered by vegetarian diets. Vegetarian products provided an additional stimulus for applications work with soy proteins. This application work differed from previous efforts, in that soy protein represented a much higher percentage of the food composition, cost was somewhat less of a consideration, and taste, while important, was not directly compared to a commonly available food standard.

The Soy Health Claim

Meanwhile, the nutrition and medical communities continue to explore links between soy protein consumption and reduced incidence of cardiovascular and other diseases, resulting in a health claim allowed by the FDA for soy protein in October 1999. The regulation permits foods that contain at least 6.25 g of soy protein per reference amount customarily consumed, as well as meeting other requirements in the regulation, to make a soy health claim (2). The allowance of the health claim initiated the possibility of an explosive growth of soy protein consumption in the United States. However, unlike previous applications of soy protein, health claim-driven applications will require (a) a high percentage of soy in the finished food, (b) equal sensory attributes compared to similar nonsoy products, (c) minimal impact on current processing, and (d) moderate cost. It remains to be seen if the necessary factors can come together to permit a full exploitation of the potential benefits of this health claim for both soy manufacturers and American consumers.

Soy manufacturers saw an increase in the interest and use of their products in early 1999, as consumer products manufacturers anticipated approval of the FDA claim. Currently, the marketplace is providing more pull for products, as consumers become educated about the benefits of soy protein by legitimate medical literature, popular medical literature, and the media (3,4) Interest in soy protein's benefits is also enhanced by the aging of the baby boomer population, who are beginning to experience the health concerns that soy protein promises to mitigate. Nearly all consumers (97%) are aware of soyfoods, and 69% of Americans recognize soyfoods as healthy, 42% report that they consume soyfoods once a month or more, and 27%

consume soyfoods weekly. In 2001, 39% of consumers were aware that soy may reduce the risk of heart disease compared to 28% in 1999 (5).

Consumers have proven themselves willing to try new foods as a measure to improve their health. An analogous situation occurred in the early 1990s, when manufacturers leapt into the low-fat market because consumers expressed a genuine interest in taking control of their health through their diet. Unfortunately, it was not long before consumers discovered that many low-fat offerings simply did not taste as good as their full fat counterparts. Manufacturers of low-fat products saw incredibly high initial product sales based on their promised product quality but very poor repeat sales. Consumers learned that despite their best intentions, they eat not just as a means to manage health, but also for pleasure.

The implications for the manufacturers of soy ingredients and finished soy-containing products are significant. To enjoy the maximum benefit from the FDA claim, the soy ingredient processors must provide consumer product developers with the soy ingredients necessary to create great tasting products. Analysis of trends from our own development projects tells us that soy ingredient manufacturers have a three- to four-year window of opportunity to provide product developers with the tools to succeed. The short time frame is more understandable in the context of the total development cycle, which is detailed later in this chapter.

The FDA provides two options to communicate soy's health benefits to consumers. The first option, and the most forthright, is provided by the health claim described above. The products that make that claim must deliver a difficult combination of high soy, low fat, cholesterol, and sodium contents, and still taste good while functioning appropriately in the manufacturing process and over the desired shelf life of the product. If the nutrient conditions are met, marketers may use a statement such as "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease. A serving of (this product) supplies ____ grams of soy protein." Thus, with this health claim approach, consumers are reminded about the 25 g/day goal for soy protein consumption and offered the possibility that meeting that goal may reduce their risk of heart disease. The basic marketing assumption here is that avoidance of heart disease will motivate consumers to try to continue to use the product. Soy manufacturers benefit when this approach is used, not only by the product's use of a high level of soy, but also by the continual reminder of the goal of 25 g/day of soy consumption.

If the combination of high soy content and other parameters is not achievable, marketers can pursue a second option that still takes advantage of the increased consumer interest by highlighting the soy content of the food via a "structure/function" claim. In that case, the product label may contain a statement about the amount of soy protein, provided that the statement is truthful and not misleading. The statement also cannot contain an express or implied nutrient content claim for soy protein. An acceptable statement in this instance is "4 grams of soy protein per serving" (2). Thus, although consumer marketers still gain access to a potentially compelling benefit that a product containing soy may imply, soy manufacturers lose the following two significant opportunities when marketers pursue this "softer" claim: (a) less soy is used in the product, and (b) consumers

are not reminded of the 25 g/day soy consumption goal. Presumably, consumer pursuit of the 25 g/day goal should drive both the largest tonnage opportunity for soy manufacturers and the greatest health benefit for the American public.

Marketers therefore make a very important decision early in the development sequence that affects the technical difficulty of a product development project as well as the size of the soy protein opportunity for manufacturers. Marketers may (a) choose to pursue development of concepts that meet the health claim, (b) choose to pursue concept development toward products that meet the structure function claim, or (c) choose to pursue both types of concepts, using the one that ultimately drives the strongest purchase interest. Obviously, pursuit of both avenues results in higher concept development costs for the marketer.

Marketers are frequently rewarded more for their judgment than their data-gathering abilities. They may choose option a or b based on their own past experiences and personal observations rather than pursuing the more costly and time-consuming third option. Thus, as marketers survey the success or failure of early product entries that utilize the health claim, their likelihood of pursuing soy health claim products will be affected. Also, as research and development teams encounter barriers to delivery of high-quality products that meet health claim constraints, they may recommend that the pursuit of a structure or function claim approach is more technically feasible.

Soy manufacturers and the health of the American population would be best served when the following conditions are met:

1. Marketers believe introduction of products utilizing the soy health claim will aid the success of a new product.
2. Research and development staffs believe such products are technically feasible.
3. Soy manufacturers can provide the appropriate ingredients when requested by the developers, and these ingredients fit the food system in question, permit familiar processing, and do not negatively impact taste, texture, color, or shelf stability. Alternately, if the soy ingredients provided impact the product or processing characteristics, soy applications personnel can provide tools to resolve the issues, requiring minimal additional effort on the part of the development teams. Or the consumer company decides to invest significant additional development resources to solve the technical issues associated with application of high levels of soy protein to meet nutrient requirements while delivering expected taste attributes.
4. The health claim is positioned in a compelling way to the consumer, inducing high trial of the product by consumers.
5. The product delivers on the promise of the positioning and fits into a consumer's lifestyle so effectively that repurchase continues, which results in business success for the product manufacturer and the soy ingredient supplier.

The Low-Carb Phenomenon

The success of low-carbohydrate diets such as Atkins (6) or South Beach (7) is driving a major shift toward low-carb food formulations. This may be a passing fad, just

like low fat diets a decade ago. Yet regardless of how long the trend lasts, it currently is having a major impact on food product consumption patterns. This low-carb approach is being delivered to consumers via three different approaches: (a) minor modifications of foods that are naturally low in carbohydrates; (b) modifications of foods, such as breads or pasta, that are naturally high in carbohydrates; and (c) introduction of “new foods,” such as bars, that meet the conditions imposed by low-carb diets. The second and third approaches generally require that the carbohydrates that might normally be used in the normal food formulation be replaced by proteins or carbohydrates that don’t “count” against the parameters of the diet. Soy protein offers an option to developers to deliver to the requirements. However, since these diets are not focused on protein or soy specifically, soy products will be used if they represent the easiest and most cost effective means to meet the technical requirements of the formulations. The feedback to soy manufacturers previously in this chapter is also relevant for low-carb opportunities.

Timetable of a Trend

When an event occurs that drives a major trend, manufacturers begin to capitalize on it. Thus, in the case of the soy health claim, the clock began ticking in earnest when the FDA approved the health claim. Savvy consumer product marketers had products in development when the claim was approved and quickly introduced them. Consumers heard the marketing messages, which increased in frequency with each new product introduction. Eventually, consumers hear enough that they understand the claim, they decide the promised benefit is one they would like to pursue, and they purchase the product. After consumption, they decide if the quality warrants a repeat purchase, this time, probably at full retail price, since there may be no coupon to drive repurchase.

These early purchases occurred in early 2000. The products were formulated with the best soy ingredients the industry had to offer in 1998 or 1999, since products often take a year to be developed, distributed, and reach the retail shelves. Since early 2000, “early adopter” consumers were trying soy products and discussing them with their friends. This word of mouth impacts trial on future new items. If the first batch of new soy products was limited by soy ingredient capabilities, these limitations will impact future product opportunities.

Now, in 2002, marketing executives are considering more new soy products. Early products appear to be “flying off the shelves,” confirming consumer’s interest. It is still too soon to determine if these products will get the repeat purchases necessary for success. As marketers get feedback from consumers, they are likely to set more stringent taste decision rules for development teams. The developers are pushing ingredient salesmen for soy products that solve problems encountered in the first round of products. We will outline specific problems encountered in several product categories later in this chapter.

Consumers and the media also have notoriously short attention spans. Soy may be a “hot item” today, but if consumers are not able to incorporate it into their diets on a sustaining basis, history shows they will quickly forget soy’s benefits and move on to a new trend. Fat free products were all the rage in the late 1980s and early 1990s, and then introductions fell off in the mid-90s as consumers failed to repeat on early introductions. Low fat products emerged as the next wave of introductions in the mid-90s, only to fall off in the late 1990s as consumers turned their attention back to more pleasurable full fat foods. Low fat claims are surging again, but this time the claims are not driven by a direct desire for low fat, but rather by the need to meet a low fat requirement to use the soy health claim (8). Reduced fat products have experienced three waves of consumer interest. It remains to be seen if the consumer interest in soy is as persistent. This sequence of events is demonstrated in Figure 8.1.

Key Issues Formulating with Elevated Levels of Soy

Amount of Soy Required

The primary challenge facing a product developer charged with making a soy-containing product that meets the FDA claim is the absolute volume of soy protein required; 6.25 g of soy protein are required per serving, which is a standardized reference amount customarily consumed (RACC) defined by the FDA for different food types. This means that soy concentration may vary widely between products. For example, a snack bar has a different serving size than bread or cereal ([Table 8.2](#)). Thus, each application and approach is different not only because food systems differ greatly, but because the target concentration of soy may also differ. Table 8.2

Products Bearing a Reduced/Low Fat Claim

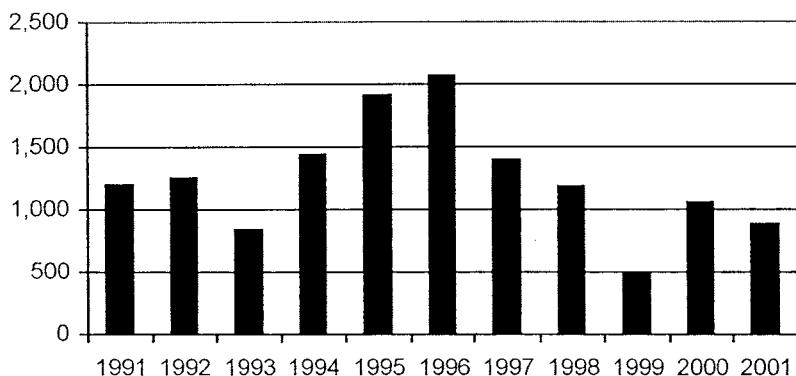


Figure 8.1. Number of new products bearing a reduced-fat or low-fat claim by year.

TABLE 8.2Examples of Reference Amounts Customarily Consumed for Relevant Food Items^a

Product Category	Reference Amount	% of Soy Protein Required to Meet Health Claim
Biscuits, croissants, tortillas, soft bread sticks, etc.	55 g	11.36
Breads and rolls	50 g	12.50
Brownies	40 g	15.63
"Heavy weight" cakes: cheesecake, fruit cakes, etc.	125 g	5.00
"Medium weight" cakes: chemically leavened cakes, cupcakes, etc.	80 g	7.81
"Light weight" cakes: angel food, chiffon cakes, etc.	55 g	11.36
Cookies	30 g	20.83
Crackers used as a snack	30 g	20.83
Grain-based bars	40 g	15.63
Beverages	240 ml	~2.6 (depending on ingredient density)
Hot cereals	1 cup prepared	~2.6 (depending on ingredient density)
Breakfast cereals	15, 30, or 55 g depending on density of cereal and other characteristics	11.36–41.67 depending on cereal
Pasta, plain	140 g prepared, 55 g dry	4.46 prepared, 11.63 dry
Legumes, beans	90 or 130 g depending on preparation, 35 g dry	6.94 or 4.81 depending on preparation, or 17.86 dry
Mixed dishes such as casseroles, etc.	1 cup prepared	~2.6 (depending on ingredient density)
Mixed dishes such as burritos, egg rolls, pizza, sandwiches, etc.	140 or 195 g depending on execution	4.46 or 3.21 depending on execution
Salads, bean or vegetable type	100 g	6.25
Snacks	30 g	20.83
Soups	245 g	2.55

^aData from Vetter, 1999 (9).

serves as an example only. Readers are encouraged to see original reference (9) or Code of Federal Regulations for details to be used in labeling and formulation.

New Forms May Be Needed

The functional benefits of soy (filming, foaming, water binding, etc.), which previously drove sales, may now be the developer's worst enemies. Developers have been forced to use existing soy ingredients optimized for various functional properties to meet the health claim instead of ingredients specially designed to suit health claim driven, end-product applications. The odds of getting the food product to perform,

meet the soy claim, and taste good would be greatly improved if a greater variety of soy ingredients with different forms and functional properties were available. Where previously soy was developed to maximize product function (maximum function at minimum level), now soy is needed that maximizes nutritional function (maximum level with minimal functional effect on the system of application). The most “functional” concentrate or isolate for some food systems may be the one with the least functionality.

The best form to deliver high soy protein levels may differ greatly by food system. The optimum situation for developers of soy health claim products may be a wider range of soy protein ingredient forms. However, this may complicate manufacturing, where long production runs of a single ingredient may be preferred to provide the lowest costs for both the ingredient supplier and the food manufacturer.

Flavor Issues Resulting from Use of High Levels of Soy Protein

Soy flavor remains a significant limitation in the acceptance of soy-containing, mainstream products (10–13). Product developers find themselves in a quandary, first working through the functional challenges that soy presents when used at high levels and then masking the off-flavors that often result. The ability to avoid or mask the soy flavor is often the difference between a market of moderate size and a huge market. There is a segment of the population that wants the health benefits of soy, but is extremely sensitive to soy off-flavor and will not repurchase products that exhibit it.

There have been four very different approaches to manage the beany flavor of soy products. The first has spawned a side business called “soy masking flavors.” The soy off-flavor problem is so profound that it has created an entire business opportunity (14–17). Flavor companies have worked to develop agents that may be added to cover the objectionable flavors from soy. Unfortunately, to date no masking agent is consistently effective across applications. Each situation is unique, and a masking approach must be developed based upon the other ingredients and the process for product manufacture.

A second approach is for marketers to limit flavors in the product line to those that work well with soy (18). For sweet product lines, fruit, acid, and chocolate flavors are quite compatible with soy, whereas vanillas often potentiate the off-flavor. This is an important concern for soy ingredients since the best-selling flavor in most product lines is vanilla or “plain.” Special care and attention must be given to getting the flavor profile right in vanilla-flavored products. If it is not right in the line-leading vanilla item, the odds of having a successful business are not good. Product trial on other flavors will likely suffer as well. Product lines based solely on non-vanilla flavors often exhibit lower trial scores on concept tests than similar product lines that contain vanilla products.

Savory flavors are often very compatible with soy; consequently, there are fewer flavor issues in main meal product lines that contain high levels of soy.

The third approach to off-flavor has been taken on by the breeders, seed designers, growers, and processors. They have been working diligently since the early

1970s to determine constituents that contribute soy off-flavors and ways in which the soy may be modified so that these flavors are eliminated (19–26). These projects are often costly, complicated, and time consuming. Suppliers have touted new “bland” soy ingredients as each new modification is made. Unfortunately, truly bland soy protein ingredients still have not quite been achieved. Soy ingredients that are perceived to be bland in one food system may still have noticeable off-flavors in another food system. Thus, further work remains in this area, despite the intensity of effort to date. However, clean-flavored functional ingredients are so important that product developers continue to eagerly await breakthroughs.

The fourth approach results because today even the blandest soy protein products have taste-driven limits on their use. If a single form of soy is used for the entire claimed amount, soy flavors are usually apparent. Using an analogy from shelf stable, acidified vegetable products, if a large quantity of a single ingredient is unpleasantly apparent in the product, use of several different forms reduces perceptibility. This forces the product developer to reach for whatever forms are available, such as powders, flakes, and puffed soy pieces, to meet the claims without incurring perceptible off-flavors. However, there are products in which certain forms such as puffed soy pieces are not consistent with the product identity. Being limited to using only one source of soy virtually guarantees a product with perceptible off-flavors.

A final, related problem results from shifts in overall product flavor profile due to flavor adsorption or alterations in flavor solubility or volatility. This problem is not caused by soybean off-flavors, but by shifts in the overall composition of the formula of the food system that result when large quantities of a new ingredient are incorporated (27–29). This shift in flavor profile may be as large an issue in development as off-flavors. If a current product is being modified to include a health claim level of soy protein, the entire flavor system may need to be reworked due to this flavor profile shift, even though no off-flavors are directly observable. Both soy protein manufacturers and flavor companies are conducting research to aid developers in resolving issues associated with flavor shifts driven by high levels of soy protein.

Manufacturers Perceptions of Soy Off-Flavors

As developers struggle to avoid soy off-flavors, manufacturers try to determine when a new soy ingredient is “good enough” to introduce. Soy manufacturers need to balance inputs including cost and impact on production. Unfortunately, most people who work on soy businesses are not particularly sensitive to soy off-flavor. People with the sensitivity usually end up working in other business areas. Thus, the people making the business decisions are often unable to perceive soy off-flavor and naturally discount its importance to developers. It is important to seek out “soy sensitive” evaluators to provide feedback on when a soy product is “bland” enough. It is also important to test new soy ingredients in a wide variety of technically different systems to determine where the ingredient delivers the bland flavor

promised. For example, soy that is bland in a high-moisture soymilk application may still provide significant off-flavor in a lower-moisture, wheat-based bread system. Accurate notation of food systems in which a particular ingredient delivers bland flavor would focus a developer's efforts on more appropriate ingredients, saving them valuable time.

Managing Functionality

In many of the products in which soy is used, it may appear that the product developer is asking for conflicting properties in the same product application. In fact, the manufacturing process used to produce the product may be very different within the same category and, consequently, have different product performance requirements.

One such example is snack bars. Four different manufacturing processes are commonly used according to the type of product desired. In some processes the products are cold-formed, so the viscosity from soy is a problem; in another processes the products are baked and the water-holding properties of the soy present a different set of problems. The key for the soy ingredient manufacturer is to understand the needs of the product and the process used.

Cost

Cost will continue to be a consideration in claim-oriented soy applications. However, manufacturers may be able to charge a higher price for products that have soy claims, making them able to afford more costly soy ingredients than previously. Normally, in traditional functionality-based applications, the cost of the soy is compared to the cost of the material it is replacing. Thus, clear cost parameters can be identified. However, in a claim-oriented application, soy is the only material that can be used. The only competition for use is from other manufacturers' soy products. Consequently, it is difficult to provide clear guidelines for reasonable cost for soy products, as each use will have different economic considerations.

In general, the manufacturer that provides the blandest, most functionally useful soy products at the lowest cost will be best positioned to succeed in this new landscape. If a significant breakthrough in soy technology will require a higher product cost, it is important to explore the benefits with customers before rejecting the opportunity due to cost. Evaluating new technology options and economics with key customers may aid manufacturers to find new compelling benefits that can command higher product costs.

Soy Protein "Tools" and Products

If developers can access the soy tools they need to truly meet consumer demands, American eating patterns may be changed for the long term to include significant quantities of soy protein. If the necessary tools are not made available, or are provided too late, the soy "craze" will follow the same path as "fat free" and "low fat", and a significant opportunity will be lost for the soy industry.

Developers Need More Soy Product Information

Product developers who embark on a project to add significant amounts of soy protein to product formulations are likely to fall into one of the following two groups: (a) scientists who are expert in the product system they are formulating (these usually work for the consumer foods company), and (b) scientists who are expert in the manufacture, structure, and function of soy proteins (these usually work for the supplier).

Often the developing scientists are not allowed to share sufficient information with the applications scientists to maximize the application opportunity. With trend-driven concepts like soy, the developing company is reluctant to share much specific information with suppliers out of fear of competitive preemption.

While developers routinely rely on applications information provided by other ingredient manufacturers (such as starch or gums), those ingredients are used for product functionality at minimum use levels in the finished product to achieve the necessary effect. In this aspect, soy applications were historically like other ingredient applications. However, once soy applications turned toward nutritional functionality, which drives use levels far above those needed for product functionality, the need of development scientists for more in-depth information increased.

Of the four major soy protein manufacturers, three currently participate in only one of the three major forms (flour, concentrate, or isolate). Consequently, each of these manufacturers provides applications literature geared to convince development scientists that some modification within their form is the best for nearly all applications. The fourth company manufactures all three forms, but provides little information to guide the scientist to the best product for an application.

If a development scientist fails in early attempts to incorporate high levels of soy into a product, then he is faced with the task of piecing together information from all the soy protein manufacturers to discern if additional options might be available to resolve his technical concerns. Few development projects permit the time necessary to find the options. Unless the food company is deeply committed to the soy concept, developers often deem the task not feasible and recommend pursuit of some concept that uses lower levels of soy. However, if sufficient information were made easily available to the developer, the original product concept could be delivered.

Current Soy Protein Products Available

Soy protein is available in three main forms: flour, concentrate, and isolate. [Table 8.3](#) outlines the relative composition of the three major soy protein products.

As is evident from the schematic in [Figure 8.2](#), there are many steps in the manufacture of each soy product, and the manufacturer has made choices in each case that impact finished ingredient performance. For example, the starting beans can vary greatly by source. Some varieties contain all the constituents expected in soy. Some are bred by traditional techniques to minimize certain potentially undesirable constituents or maximize others. Other varieties are modified via genetic engineering. The fat can be removed from flaked beans by solvent or mechanical extraction. The fat can be re-

TABLE 8.3Composition of Soy Protein Products^a

Component	% (as-is basis)		
	Flour	Concentrate	Isolate
Composition description	Full composition of soybean, less fat; includes sugars, fiber, minor constituents, and protein.	From soy flour, removing sugars, may also remove minor constituents depending on process; protein and fiber retained.	From soy flour, removing sugars and fiber; protein and minor constituents retained, depending on process.
Protein (N × 6.25)	52–54	62–69	86–87
Fat (pet. ether)	0.5–1.0	0.5–1.0	0.5–1.0
Soluble fiber	2	2.5	<0.2
Insoluble fiber	16	13–18	<0.2
Ash	5.0–6.0	3.8–6.2	3.8–4.8
Moisture	6–8	4–6	4–6
Carbohydrates (by difference)	30–32	19–21	3–4

^aData from Endres (31).

moved to varying degrees, or some may be added back later in processing as either fat or lecithin. Flours can be ground to a variety of particle sizes. Soy concentrate can be extracted using acid, aqueous alcohol, or moist heat and water, which greatly affects the minor constituents contained in the concentrates. Isolates may be sold as “isoelectric isolates” or may be neutralized. Flours, concentrates, and isolates may be treated with heat or mechanical work to varying degrees to increase solubility and functionality. Some products are partially hydrolyzed to enhance whipping characteristics. These products may also be extruded to texturize them into fibers or chunks.

Each processing step alters the functional properties of the soy ingredient and adjustments may provide an opportunity to resolve important applications problems for the food developer. If a more complete schematic could be developed outlining the processing steps, the options available at each step, the specific changes in material achieved, and some indication of the economic implications, communications between the manufacturer and development scientists would be greatly enhanced. Such communications would enhance the odds that desired products containing high levels of soy could be delivered.

Barriers in Specific Application Categories

Beverages

Beverages represent an extremely large market with many opportunities for soy. The standard serving size for a beverage is 240 ml, so incorporating 6.25 g of soy protein

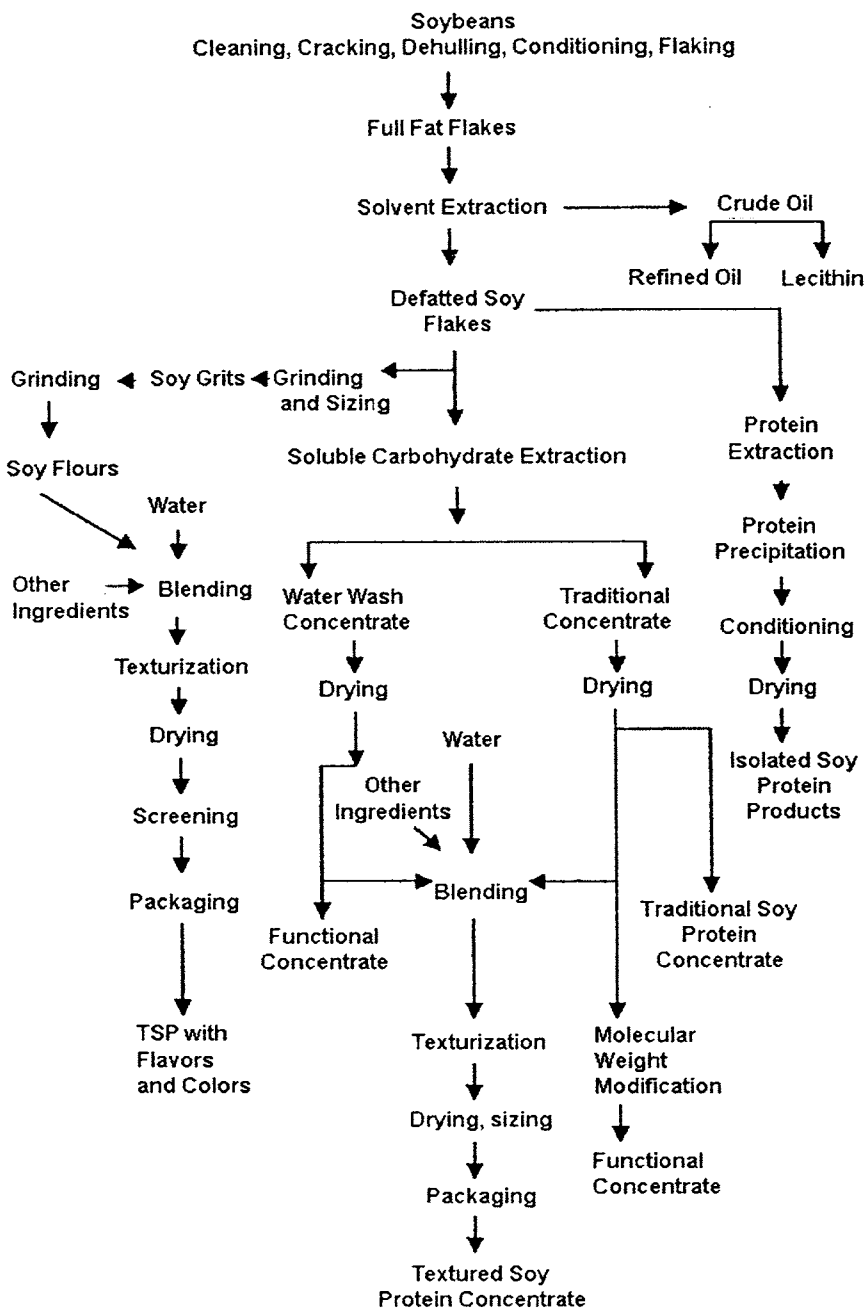


Figure 8.2. Key steps in the manufacture of soy protein products.

into that large volume of product is not particularly difficult. However, formulators are currently limited by the ingredients available to use in opaque beverages, products with relatively high viscosity like smoothies, those tolerating chalky textures, or having strong flavors. New markets could be available if soy ingredients were available that could be used in clear beverages, beverages with lower viscosity, in vanilla or plain flavored beverages, and soy without chalky, astringent texture.

Flavor continues to be the major issue for soy products used in beverages. As discussed previously, the line leading flavors tend to be vanilla or plain flavors, which provide little opportunity to cover any off-flavors present. Soy-containing beverages are usually recommended to be served cold, which diminishes the perception of soy off-flavors. In applications in which the beverage is usually served cold, but may also be served or used hot, the flavor may be acceptable cold and very unacceptable hot. This problem with a minor use occasion can also impact repeat sales for a product.

Other frequent issues relate to color, settling, and foaming. Soy proteins tend to contribute an off-color to an opaque beverage, as opposed to the bright white color consumers expect from dairy proteins or clouding agents. It is very difficult to mask the off-color, and darker colors can cue off or overcooked flavors even when they are absent. The nonwhite color may therefore exacerbate expectations of an off-flavor concern.

Settling disturbs reliable delivery of the desired drink texture, as consumers may not always shake the product before consumption or they may do so inadequately. It also prevents a beverage from being sold in a clear container, which may be desired by marketing. Finally, settled proteins often contribute a chalky texture that might not be present if the proteins were properly dispersed. However, for reasons that are not apparent based on the information provided, a product developer is sometimes faced with a trade-off between improved solubility or dispersibility and better flavor. This trades off one desirable attribute with another and usually results in a compromised product.

Some products also exhibit foaming characteristics, which may be desirable for some product concepts and very problematic for others. Foaming causes major problems in manufacturing or food service applications.

Manufacturers are currently introducing products that claim significant enhancement in needed beverage properties. Time will tell if the new soy products provide sufficient improvements to permit soy-enhanced beverages to find their way into mainstream American beverage consumption.

Baked Goods

Both the opportunities and difficulties in the baked goods segment may be larger than one would suspect. The reason is that the soy protein must be used at such a high percentage level to make the FDA's claim. For example, bread must contain 12.5% soy protein by weight, whereas bagels, biscuits, and tortillas must contain

more than 11%, and crackers and dry mixes, such as nutbreads, more than 20% by weight.

In these food systems, the water holding of soy protein affects the batter viscosity and dough consistency. The increased viscosity may be countered by increasing water to match current viscosity, resulting in a yield improvement. However, once the dough or batter is baked, the finished product may have significantly higher moisture content. This can shorten mold-free shelf life and may also alter the aging characteristics of the finished baked good. Unfortunately, the alterations are not consistently beneficial. For some bread products, the increased moisture content of the finished product may delay staling-type changes. For others, higher moisture may cause coarse product grain, which often stales faster. For chemically leavened products, the additional water in the finished product may alter the rate of firming, development of fragility, and flavor losses, either increasing or decreasing rates depending on the specifics of the system.

If increased water holding in dough or batter is not countered by some means, the machining properties of the dough or batter are often sufficiently changed to necessitate major processing changes.

When high levels of soy are added, wheat gluten is diluted, and product volume is often affected. If fortifying vital wheat gluten is added to counterbalance the dilution, this adds cost to the product. It may also further darken a crumb that is already darkened by the addition of soy, which is a serious negative in many bread, roll, and cracker products.

Bland, characteristic flavor is essential in products such as white bread. Unfortunately, soy products promising bland flavor are often evaluated in systems other than bread, and when tested in bread, they may contribute unexpectedly high off-flavor.

Finally, pH may be a problem. Since many soy proteins are neutralized and soy protein has significant buffering capacity of its own, the pH of wheat-based products can be altered. This can cause problems in mixing by altering the pH of the dough, which alters the mixing behavior of wheat proteins, and by altering acid consumption in chemical leavening systems, thus altering the timing of CO₂ generation. This can also cause problems in the finished products by altering the pH environment, which can shift flavor component volatility, and by impacting the efficacy of antimycotic systems that are very pH sensitive for activity.

Grain-Based Bars

Grain-based bars are frequently used as a vehicle for soy protein. In 2001, of 164 items listed in the Global New Products database that included soy protein isolate on the ingredient declaration, 43% were bars (30). However, not all of these products attempted to meet the soy health claim.

Bars differ from many baked goods due to their low moisture content, which eliminates concerns about pH, which are driven primarily by the need for mold inhibition. Bars also do not depend on wheat gluten for their structure. Both of these

facts should make bars a much easier application vehicle for health claim levels of soy than baked goods.

However, several difficult concerns remain. Because the reference serving size for the bars is less than for most baked goods, there is a higher resultant soy protein percentage in the finished product. For bars made by cold processes, the water-absorbing capabilities of soy protein can be problematic, by increasing the viscosity of the forming matrix. For bars that are baked, the water-absorbing characteristics continue to be a problem during forming, and the water-holding characteristics may slow moisture loss during baking, making it difficult to remove sufficient water to meet low water-activity requirements.

Soy protein can be delivered to bars in a variety of forms, such as powders, grits, and pieces, which potentially eases the problems of meeting flavor and texture objectives. However, the behavior of these forms over shelf life may be a problem, because the bars may harden or become more friable over time. The stability knowledge gained by a manufacturer in previously introduced bar product lines can be greatly altered by the addition of significant amounts of soy protein, as soy protein interacts with the water, fat, carbohydrate, and other proteins present in the bars. This often means that a complete shelf life study may be required before the new bar product can be prudently introduced, which may greatly lengthen the total development time required.

Breakfast Cereals

Breakfast cereals are available with soy protein, but currently no mainstream items have levels necessary to make the health claim. The reference amount of cereal per serving varies depending on characteristics of the cereal, but again, to make the health claim a relatively high weight-percent must come from soy protein. Taste, texture, and process compatibility are impediments to commercializing products with soy at high levels. Off-flavors, water binding, alteration of machining properties, and impact on texture, bowl-life, and shelf life are all-important issues in dry breakfast cereal manufacture.

Hot cereals present a smaller challenge. Since the product has high moisture content as consumed, more approaches are available to manage the higher water-holding capacity of soy protein. Since these products are usually not made on high-speed extrusion lines, there is less processing impact due to adding protein to a largely starch-based system.

The warm serving temperature of hot cereals can magnify the off-flavor problem by increasing the volatility of the off-flavor compounds. So although the processing and textural issues are more manageable in hot cereals, soy use levels may be capped by the flavor problem.

This is a category where marketers appear to have largely backed away from health claim levels of soy and have moved to structure and function claim levels. While this still facilitates soy sales, there could be further opportunities if the quality issues could be addressed.

Soups, Side Dishes, and Entrees

Savory flavors are generally compatible with soy. In addition, the weight per serving for soup, side dish, and entree categories is larger than for bread, cereals, and so on, so health claim levels of soy comprise a lower percentage of the total product. However, inclusion of soy can lead to a product being perceived as a vegetarian item. The issue here is to formulate great tasting items that are marketed by mainstream manufacturers in a way that appeals to mainstream consumers.

Soy can be included as textured product in partial or total replacement of meat. It can be incorporated into some of the meal components, such as pasta or sauce, or it may be included directly as a whole bean. Since soy can be worked into the food in several ways, it is more feasible to circumvent texture, processing, and stability concerns in these systems.

Since soy flour and whole soybean products are more likely to be used in this category because of feasibility and cost, the importance of potential gastrointestinal side effects should not be ignored. Flatulence is generally attributed to the fact that humans do not possess the enzyme α -galactosidase, necessary for hydrolyzing the α -galactosidic linkages of raffinose and stachyose to yield readily absorbable sugars (31). Most normal varieties of soybeans contain these oligosaccharides, but newer varieties are being introduced that reduce or substantially eliminate these sugars.

As we have learned from other categories, most notably fat alternatives such as Olestra, digestibility problems for any family member may cause all family members to stop purchasing the product. While it may be more feasible to address quality concerns in this category while delivering health claim levels of soy, if digestibility issues are not also addressed, the product will ultimately fail.

Snacks

Soy nuts are currently sold as an alternative to dry roasted peanuts, but only usually in limited distribution at specialty outlets. They are currently positioned as a specialty and not a mainstream item. Manufacturers may want to give thought to how soy might be incorporated into snacks that are already familiar to the public.

Soy protein can also be incorporated into more traditional snacks, but there will be significant difficulties meeting requirements for the health claim. Since the serving size for snacks is 30 g, the required 6.25 g of soy protein represents more than 20%, by weight, of a formulated snack. Use of soy protein in tortilla chips or extruded snacks is often limited by soy's effect on dough behavior and moisture loss during baking. Use in other snack types presents similar problems. Snacks represent a category in which soy may be incorporated at lower levels and promoted in other ways than use of the health claim.

The health claim also constrains fat and sodium levels in products that make health claims, further increasing the difficulty of development of acceptable snack products. If a strategy is devised to address the fat and sodium concerns, the technical concerns associated with incorporating soy into these systems would be similar

to those outlined for baked goods, bars, and cereals, depending on the particular snack product under development.

Individual Versus Family Products

Another consideration for soy-containing products is that of the individual serving size, not just family-sized products. If only one family member is truly in tune with the health benefits and really likes the product, they could make a purchase without the risk of waste. Also, in our experience, since soy flavor objectors occur in roughly 15% of the population, there are reasonable odds that one family member may have a strong dislike for soy-containing products if the off-flavor is present. Since a dissenting family member often stops a product's purchase, family-sized soy-containing products may be subject to this phenomenon. Examples of this problem are seen in sales of chocolate chip cookies (cookies with nuts always sell in lower volume than those without), oatmeal raisin cookies versus oatmeal alone (there are raisin-haters), and side dishes with red peppers (many children do not like red peppers).

Gastrointestinal side effects of soy carbohydrates are mainly a concern for soy flour and whole soy products if beans are used that contain raffinose and stachyose. Although low levels of soy may not have triggered this concern in earlier applications, the high levels needed to meet the health claim may. If only one family member suffers from this effect, it may be enough to discourage future purchases of family use products. Developers have two approaches to manage this issue: avoid use of soy forms that trigger the problem, or package those products in individual serving formats to focus the usage on those who are unaffected.

Procurement Trends

The opportunities for soy discussed in this chapter may result in more heavy usage of soy by companies that previously did not use it or purchased only small amounts. When soy moves from a minor to an important ingredient for a company, new considerations may emerge.

Sole Sourcing

When soy is a minor ingredient, it may be acceptable to purchase all needed quantities from a single supplier or location, and deal with interruption in supply only when the problem occurs. However, when it becomes a key ingredient in products, a company may insist on multiple suppliers or locations to manage the risk of supply interruption. Since, in these cases, soy is used at a high level and has significant effects on the product's performance, it may be difficult to find an exact match from a second source. Even a second manufacturing location for the same manufacturer may have a product with the same specifications that does not perform in exactly the same way due to minor differences in raw material sources, manufacturing processes, and so on. It may be necessary to determine the difficulty of finding an

alternate source for a soy product, and develop plans for product lines that manage the specific concerns encountered.

Ingredient Consolidation

Many companies have policies to consolidate similar ingredients wherever possible to manage inventory and logistics issues. However, many of the approaches described in this chapter may result in use of a variety of relatively similar soy products to solve specific development issues. It may be necessary to temporarily increase the number of soy products purchased to meet development objectives. Once experience is gained in a new soy application, approaches may be identified that will permit consolidation of some relatively similar soy ingredients back to a common form.

Allergen Scheduling

The FDA has included soy as one of eight categories of ingredients that are generally agreed to cause serious allergic reactions in some individuals. Manufacturers are responsible for ensuring that food is not adulterated or misbranded as a result of the presence of undeclared allergens (32).

In response to this situation, some food companies are considering allergens in manufacturing scheduling. The decision to add soy to foods that previously did not contain it will therefore impact scheduling and manufacturing beyond the formulation and processing changes themselves. If a manufacturer is using soy in a product or a manufacturing facility for the first time, appropriate measures should be taken to manage any allergen concerns.

Suggestions for Future Directions

The American diet will be enhanced if food scientists succeed in formulating conventional foods that incorporate significant levels of soy protein products. To accomplish this goal, the soy protein-supplying industry must continue to focus on several critical areas: (a) continuing to develop soy protein products that are bland under the conditions of use; (b) providing a wide variety of functional properties, again focusing on the conditions of use; (c) recognizing that the best functionality for some applications may be quite different from traditional definitions of functionality, as described in this chapter; (d) providing data to developers that permit an easy and comprehensive comparison of protein materials available, and, if possible, to standardize the information so that comparison can be made across various manufacturers' materials and so that the information will facilitate the selection of the best ingredient for a particular application and will also clarify to both the scientist and the supplier when a material cannot perform requested functions in a food product; and (e) continuing to focus on the most cost-effective soy protein products possible.

References

1. Anonymous, *The Protein Book*, Central Soya Company, Inc., Fort Wayne, Indiana, 1998, p.1.
2. Anonymous, *A Guide to Using the Soy Health Claim to Market Soy Products*, Cargill, Inc., Cedar Rapids, Iowa, 2000, p. 3.
3. Anonymous, *Chemical Market Reporter*, Vol. 258 (Suppl.), pp. 8, 10, 12, 14 (Sept. 25, 2000).
4. Anonymous, *Performance Chemicals Europe*, Vol. 16, No. 2, p. 27 (Mar. 12, 2001).
5. United Soybean Board, National Report 2001–2002, *Consumer Attitudes About Nutrition*, p. 3.
6. Atkins, R.C., *Dr. Atkins' New Diet Revolution*, American Bar Association, 2002.
7. Agatston, A., *The South Beach Diet*, Rodale Press, Inc., 2003.
8. Dornblaser, L., *Global New Products Database*, Mintel Corporation, Chicago, 2002.
9. Vetter, J.L., *Food Labeling—Requirements for FDA Regulated Products*, American Institute of Baking, Manhattan, KS, 1999, pp. F2–F12.
10. Goossens, A.E., Protein Food—Its Flavours and Off-flavours, *Flavour Industry* 5(11/12):273–274, 276 (1974).
11. Goossens, A.E., Protein Flavour Problems, *Food Processing Industry* 44(528):29–30 (1975).
12. Kinsella, J.E., and S. Damodaran, *Flavor Problems in Soy Proteins: Origin, Nature, Control and Binding Phenomena*, pp. 95–131 (1980).
13. Ovenden, C., Some Problems of Flavouring Fabricated Foods, *Food Technol. Aust.* 32:558–563 (1980).
14. LaBelle, F., Flavors Banish Beany Notes, *Prepared Foods*, Sept. 2001.
15. Brandt, L.A., Flavor Masking: Strategies for Success, *Prepared Foods*, July 2001.
16. Turner, D., Beverages for Bounty, *Food Product Design*, July 2001.
17. Granato, H., Masking Agents Maximize Functional Foods Potential, *Natural Products Industry Insider*, Feb. 27, 2002.
18. Swartz, W.E., *et al.*, Use of Soy Products Having a Reduced Beany Flavor in Meat and Other Food Products, U.S. Patent 4556571, 1985.
19. Kon, S., *et al.*, pH Adjustment Control of Oxidative Off-Flavors During Grinding of Raw Legume Seeds, *J. Food Sci.* 35:343–345 (1970).
20. Lao, T.B., A Study of the Chemical Changes Relating to Flavor of Soybean Extracts, *Dissert. Abstr. Int. Sec. B. Sci. Eng.* 32:5858–5859 (1972).
21. Greuell, E.H.M., Some Aspects of Research in the Application of Soy Proteins in Foods, *J. Am. Oil Chem. Soc.* 51:98A–100A (1974).
22. Chiba, H., *et al.*, Enzymatic Improvement of Food Flavor. II. Removal of Beany Flavor from Soybean Products by Aldehyde Dehydrogenase, *Agric. Biol. Chem.* 43:1883–1889 (1979).
23. Kim, S.-D., *et al.*, A New Beany Tasteless Soybean Variety “Jimpumkong 2” with Good Quality, *RDA J. Crop Sci.* 39:112–115 (1997).
24. Samoto, M., *et al.*, Improvement of the Off-Flavor of Soy Protein Isolate by Removing Oil-Body Associated Proteins and Polar Lipids, *Biosci. Biotechnol. Biochem.* 62:935–940 (1998).
25. Maheshwari, P., *et al.*, Off-Flavor Removal from Soy-Protein Isolate by Using Liquid and Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc.* 72:1107–1115 (1995).

26. Zhou, A., and W.L. Boatright, Precursors for Formation of 2-Pentyl Pyridine in Processing of Soybean Protein Isolates, *J. Food Sci.* 65:1155–1159 (2000).
27. Aspelund, T.G., and L.A. Wilson, Adsorption of Off-Flavor Compounds onto Soy Protein: A Thermodynamic Study, *J. Agric. Food Chem.* 31:539–545 (1983).
28. Crowther, A., *et al.*, Effects of Processing on Adsorption of Off-Flavors onto Soy Protein, *J. Food Proc. Eng.* 4:99–115 (1980).
29. Fujimaki, M., and S. Honma, Determination of Off-Flavor Compounds Absorbed in Soy Protein Isolate, *Nutritional Science of Soy Protein* 2:14–18 (1981).
30. O'Donnell, C.D., Ingredients in Use: Soy Protein, *Prepared Foods*, Feb. 2002, p. 21.
31. Endres, J.G., *Soy Protein Products*, AOCS Press and the Soy Protein Council, Champaign, Illinois, 2001.
32. Food and Drug Administration, Sec. 555.250 Statement of Policy for Labeling and Preventing Cross-contact of Common Food Allergens, *Compliance Policy Guide Office of Regulatory Affairs*, Aug. 2000 edition, updated April 19, 2001, p. 1.

Chapter 9

Value-Added Products from Extruding-Expelling of Soybeans

Tong Wang, Lawrence A. Johnson, and Deland J. Myers

Iowa State University, Ames, IA 50011

Increasingly, extruding-expelling (E-E) plants, often referred as “mini-mills,” are being constructed by farmer-owned businesses to process soybeans produced in local areas. E-E processing is a mechanical process that has several advantages over conventional processing methods. E-E mills, most employing the Express System® (Insta-Pro Div., Triple “F”, Inc., Des Moines, IA), are relatively small, with capacities ranging from 6 to 120 tons/day. They have low initial capital investment (\$150,000–200,000) and relatively low operating costs (\$25/ton) (1). E-E mills are especially well suited for processing identity-preserved (IP) soybeans. The large-scale solvent extraction (SE) facilities, which have typical crushing capacities of 2,000 to 3,000 tons/day, are not feasible for flexible IP processing. Usually, there is low production tonnage during the developmental stages of these seeds, and a large number of value-added traits are being developed. Recent stringent environmental laws also often restrict construction of new SE plants, and E-E mills can be an alternative. Because E-E products are not treated with chemical solvents, the crude oil and meal may be considered to be “organic” or “natural,” if appropriate methods are used during soybean production and further processing. Currently, the partially defatted soybean flour (about 6% residual oil) produced from these operations is not extensively used in food applications due to limited technical information on protein functionality and on performance in food applications. Some of the potential applications include baking, meat extending, animal feeding, and producing industrial soy protein-based adhesives. This chapter summarizes the recent efforts aimed at improving E-E processing and developing applications for E-E protein products.

E-E Process

In E-E processing, dry extrusion is used as a shearing and heating pretreatment to disrupt the cellular organization of the seed and free the oil. An expeller or screw press is then used to press out the oil. The extruder, as used for many years in the food industry, consists of a flighted screw that rotates in a tight-fitting barrel to convey and compress the feed material, which is pressed into a dough-like material. As the material progresses toward the die, both temperature and pressure increase as a

result of the relatively shallow screw flights and increased restriction. The sudden pressure drop as the product is forced through the die causes expansion of the extrudate. Entrapped water vaporizes or “flashes off” due to the high internal temperature. All of these events cause disruption of cell walls and subcellular organizations and denaturation of proteins, and free the oil held in spherosomes.

Dry extrusion processing of soybeans was developed in the 1960s to enable Midwestern U.S. soybean growers to cook soybeans for use as livestock feed right on the farm where the soybeans were produced (1). The process uses friction as the sole source of heat to deactivate the antinutritional factors present in oilseeds. This type of extruder typically uses a three-segment screw with intervening steam or shear locks to prevent backflow of steam and molten product and to increase shear. The product prepared from whole soybeans is a dry extrudate with an average of 38% crude protein and 18% oil, and has been successfully used in high-energy diets for livestock. On the other hand, continuous screw pressing (SP) or expelling, the major soybean processing technique before World War II, had relatively low oil-removal efficiency, leaving 4–8% residual oil (RO). This mechanical method was largely replaced by SE.

Coupling dry extrusion and expelling was first reported by Nelson *et al.* (2) at the University of Illinois for processing soybeans to obtain good quality oil and meal high in protein. A process flow diagram for E-E processing is shown in [Figure 9.1](#). In the method of Nelson *et al.* (2), the coarsely ground whole soybeans with 10–14% moisture content were extrusion cooked. The residence time in the extruder was less than 30 seconds, and the internal temperature was about 135°C. The extrudate that emerges from the die was a hot semi-fluid and was immediately pressed in a continuous screw press. Extruding prior to SP greatly increased the throughput of the expeller. About 70% oil recovery was obtained in single-pass expelling. Press cake with about 50% protein, 6% RO, and 90% inactivation of trypsin inhibitor (TI) was obtained from dehulled soybeans. The high-temperature, short-duration heat treatment of extrusion successfully replaced prolonged heating and holding of raw materials as practiced in conventional SP operations.

Bargale *et al.* (3) also used E-E processing to process soybeans. Three different types of extruders and processing conditions were used to enhance oil recovery. Pressing variables, such as pressure, temperature, and sample height, were studied using a hydraulic press. Over 90% of the available oil could be recovered by using extrusion as pretreatment for batch pressing.

Qualities of Meals and Oils Produced by E-E, SP, and SE

Soybean oil and meal produced by E-E processing have unique characteristics compared with products produced by SE. Wang and Johnson (4) compared quality characteristics of oils and meals produced from different types of soybean processing methods. Soybean oil and meal samples were collected three different times over a one-year period from 13 E-E mills, eight SE plants, and one continuous SP plant. The quality characteristics of the soybean meals are presented in [Table 9.1](#). SP was

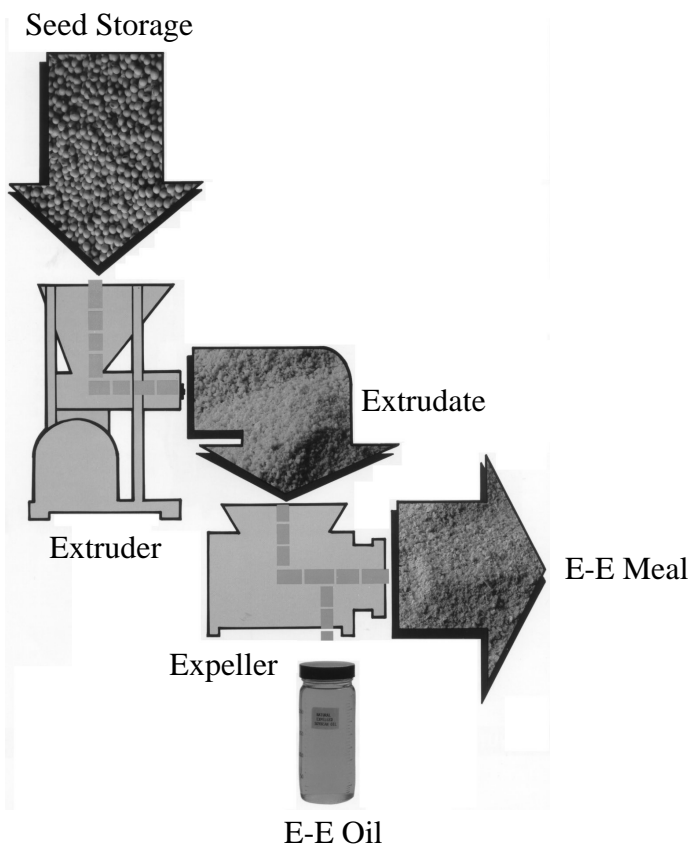


Figure 9.1. Extruding-expelling (E-E) system used for soybean processing (adapted from Insta-Pro International product brochure).

slightly more efficient in recovering oil than was E-E processing, leaving 6.3% oil compared with a mean of 7.2% for E-E meals. These values were considerably higher than those for SE meals (1.2%).

The degree of protein denaturation in soybean meal is typically measured by determining protein solubility under alkaline (KOH) conditions, urease activity, and protein dispersibility index (PDI). KOH protein solubilities of E-E and SE meals were not significantly different, nor were urease activities, indicating that the amounts of heat exposure for feed purposes were equivalent. SE meals had an average of 61.6% KOH protein solubility and 0.03 pH units of urease activity, suggesting much greater protein denaturation. PDI values of E-E meals (mean of 18.1) were much lower than those of the SE meals (mean of 44.5), indicating higher degrees of protein denaturation were achieved in E-E processing. Relationships between PDI and KOH protein solubilities were different between E-E and SE meals ([Fig. 9.2](#)).

TABLE 9.1

Quality Characteristics of Soybean Meals Produced by Extruding-Expelling (E-E), Solvent Extraction (SE), and Screw-Press (SP) (4)^a

	E-E	SE	SP
Moisture, %	6.9 b	11.7 a	11.0 a
Oil, % ^b	7.2 a	1.2 b	6.3 a
Protein, % ^b	42.5 b	48.8 a	43.2 b
Fiber, % ^b	5.4 a	3.7 b	5.9 a
Urease, ΔpH	0.07 a	0.04 a	0.03 a
KOH solubility, %	88.1 a	89.1 a	61.6 b
PDI ^c	18.1 b	44.5 a	10.6 c
Rumen bypass, %	37.6 b	36.0 b	48.1 a
Trypsin inhibitor, mg/g	5.5	5.5	0.3

^aThe values in the same row with different letters are significantly different at 95% confidence level.

^bPercentages are based on 12% moisture content.

^cProtein Dispersibility Index.

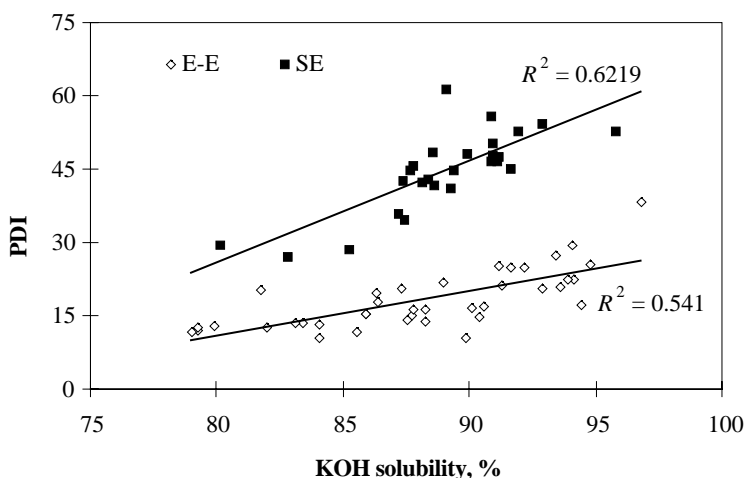


Figure 9.2. Relationship between protein dispersibility index (PDI) and KOH protein solubility of soybean meals (4).

Rumen-bypass or rumen-undegradable protein (RUP) is an important measure of potential protein utilization by ruminant animals. A higher RUP indicates that more protein will escape rumen bacterial fermentation and will be utilized by the animals. An ammonia-release procedure was used for RUP determination (5). RUP values were similar for E-E and SE meals (37.6 versus 36.0%, respectively), which have different degrees of protein denaturation as measured by PDI. [Figure 9.3](#) shows a scatter plot of RUP versus PDI. E-E meals, which had more protein denaturation

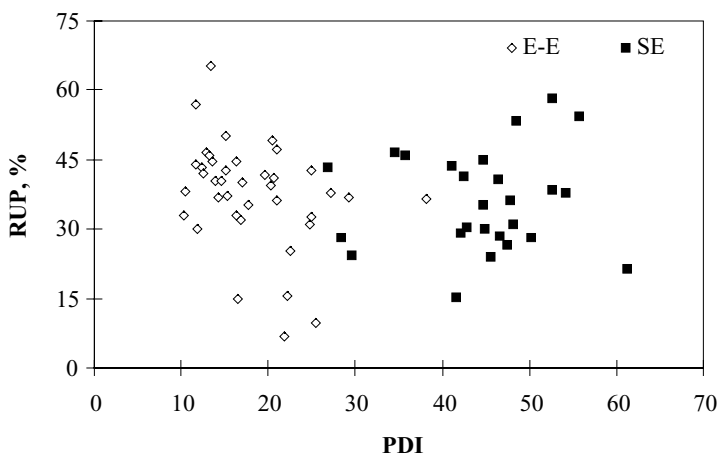


Figure 9.3. Relationship between protein dispersibility index (PDI) and rumen undegradable protein (RUP) of soybean meals (4).

than SE meals (as shown by low PDI), should have had higher RUP values. But the very brief heat exposure of E-E processing (about 30 seconds) at low moisture content may not have produced the kind of protein denaturation needed to pass the rumen intact. It is common practice to hold the beans at elevated temperatures after roasting to allow more thorough heat treatment in order to produce feed ingredients with high RUP for lactating dairy cows. By carefully examining the scatter plot, a general trend could be identified. There seemed to be a minimum RUP value at a PDI value of approximately 30. Below this PDI, the lower the PDI, the higher the RUP values; above this PDI, the higher the PDI, the higher the RUP values. When inadequately denatured, the protein may not be readily available to rumen bacteria; therefore, a higher percentage of the protein passes through the rumen.

TI activity is an important quality parameter of soybean meal, especially when the meal is fed to monogastric animals. Urease activity is usually used as an indicator for TI activity. There are no differences in urease activity or TI activity between E-E and SE meals, and the low values suggest that the antinutritional factors have been sufficiently inactivated.

The essential amino acid compositions of soybean meals processed by different methods are shown in [Table 9.2](#). Arginine, cysteine, and lysine percentages in SP meal were considerably lower than for the soybean meals processed by other processing methods, suggesting degradation of these amino acids under severe heat treatment. Heating generally increases digestibility of amino acids. But when exposed to excessive heat, the amino acid digestibility could be reduced, especially for lysine and cysteine (6). The amino acid composition data in this report are similar to those of Baize (7).

The qualities of E-E, SE, and SP soybean oils are compared in [Table 9.3](#). Peroxide value (PV) is a measure of primary lipid oxidation products in the oil. The

TABLE 9.2

Essential Amino Acid Compositions of Soybean Meals in Percent of Total Protein (4)^a

Amino Acid	E-E	SE	SP
Arginine	7.45 a	7.56 a	7.27 b
Cysteine	1.73 a	1.60 b	1.51 b
Histidine	2.77 a	2.76 a	2.75 a
Isoleucine	4.64 ab	4.54 b	4.70 a
Leucine	7.92 b	7.92 b	8.03 a
Lysine	6.50 a	6.49 a	6.01 b
Methionine	1.49 ab	1.48 b	1.54 a
Phenylalanine	5.18 a	5.15 a	5.21 a
Tyrosine	3.60 a	3.59 a	3.60 a
Threonine	3.94 a	3.97 a	4.01 a
Tryptophan	1.47 a	1.44 a	1.45 a

^aThe values in any row with different letters are significantly different at 5%.

TABLE 9.3

Quality Characteristics of Soybean Oils Produced from Extruding-Expelling (E-E), Solvent Extracting (SE), and Screw Press (SP) (4)^a

	E-E	SE	SP
PV, meq/kg	1.73 a	0.96 b	1.76 a
FFA, %	0.21 b	0.31 ab	0.33 a
Phosphorus, ppm	75 c	277 b	463 a
AOM ^b stability, h	23.9 b	39.8 a	36.2 a
Moisture, %	0.08 a	0.08 a	0.05 b
Tocopherols, ppm	1257 b	1365 a	1217 b
Color, red	10.2 b	11.1 b	17.5 a

^aThe values with different letters in the same row are significantly different at 95% confidence level.

^bActive oxygen method.

PVs of the crude E-E oils (mean of 1.73 meq/kg) were significantly higher than those of crude SE oils (mean of 0.96 meq/kg), which was attributed to the high temperature used in the E-E process, the long period allowed for oil cooling, and/or the poor oil storage conditions and longer storage times at the E-E mills. Crude SP oil (1.76 meq/kg) had a similar PV as the mean for E-E oils. Free fatty acid (FFA) content is a measure of hydrolytic degradation during seed storage and oil extraction, and higher FFA values result in higher refining losses during subsequent oil refining. The FFA contents of E-E processed oils (mean of 0.21%) were significantly lower than those of SE oils (mean of 0.31%), which may be due to the rapid inactivation of lipases during extrusion. SP oil contained 0.33% FFA, which was similar to that of SE oils.

Phospholipids (PLs), also referred to as gums or lecithin, are polar lipids in the oil. PL contents of the oils after natural settling were much lower in E-E oils (mean of 75 ppm phosphorus) than in SE oils (mean of 277 ppm phosphorus). SP oil had much higher PL content (463 ppm phosphorus) than did SE oil. The PLs in E-E oils were more hydratable and easier to settle; these properties were attributed to the rapid heat inactivation of the phospholipases. Tocopherols are a group of natural compounds possessing antioxidant activity. Their concentration and composition influence the oxidative stability of the oil. Total tocopherol contents of the E-E oils were slightly, but statistically and significantly, lower than those of the SE oils (mean of 1,257 versus 1,365 ppm). Oxidative stabilities, as measured by the active oxygen method (AOM), of the E-E oils (mean of 23.9 hours) were significantly lower than those of the SE oils (mean of 39.8 hours), probably due to the higher PVs and lower contents of phosphorus and tocopherol in E-E oils. The AOM value of the SP oil (mean of 36.2 hours) was greater than that of E-E oil due to its higher PL content, but less than that of the SE oils. The colors of the E-E (mean of 10.2 red) and SE (mean of 11.2 red) oils were not statistically different, although SE oils tended to be slightly darker than E-E oils. SP oil (17.4 red) was much darker in color than the other two types of oils, probably due to the more severe heat treatment before pressing.

Characteristics of E-E Meals Produced under Various Processing Conditions

Currently, the partially defatted E-E soy flour (ground E-E meal) is not extensively used in mainstream food products, because little information is available about its functionality and potential in food applications. One potential use of partially defatted soy flour is the production of texturized vegetable protein (TVP). However, it is believed that partially defatted soy flour will perform much differently in TVP production than the traditionally defatted soy flours because of the extensively heat-denatured protein and high oil content. Crowe *et al.* (8) and Heywood *et al.* (9) studied the range of PDI and residual oil content that could be produced by E-E processing, and characterized the functionalities of these partially defatted soy flours.

In the Crowe *et al.* study, soybeans were processed using an Insta-Pro 2500 extruder and an Insta-Pro 1500 screw press (Insta-Pro Div., Triple "F", Inc., Des Moines, IA). The extruder temperature was adjusted by manipulating the screw design and shear-lock configuration, as well as the die (nose cone) restriction. SP conditions were modified by changing choke settings. Partially defatted soy flours having a wide range of PDI values (12.5 to 69.1) and RO contents (4.7 to 12.7%) were achieved by changing extruder and SP operating conditions. The relationships between residual oil (RO) content and PDI, and between extruder temperature (zone 1, the highest temperature region) and PDI or TI activity are shown in [Figures 9.4](#) and [9.5](#). PDI correlated with RO content and extruder temperature.

TI activities ranged from 4.5 to 97.5% of the activity of raw soybeans and decreased with increasing extruder barrel temperature. Guzman *et al.* (10) varied

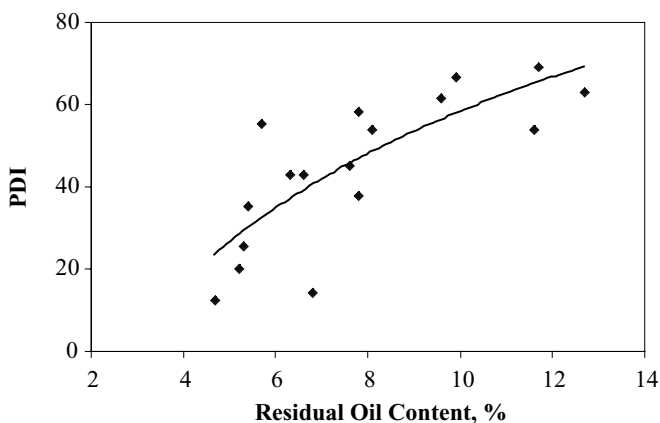


Figure 9.4. Relationship between protein denaturation (PDI) and residual oil content of E-E meals (8).

extrusion temperatures from 127 to 160°C and reported that residual TI activities in non-expelled samples were between 2 and 31% of the original activity. The activities of all three lipooxygenase isozymes (L1, L2, and L3) decreased with increasing temperature and were not detectable in most of the partially defatted soy flours when the extruder temperature was greater than 89°C (8).

Functionalities of E-E Flours Produced under Various Processing Conditions

The low-fat soy flours (LFSF) obtained as described above can be grouped into three PDI/RO categories: low PDI/RO ($14.3 \pm 5.0/6.8 \pm 0$, designated as low LFSF), mid-range PDI/RO ($41.6 \pm 3.0/7.8 \pm 1.8$, mid LFSF), and high PDI/RO ($66.6 \pm 4.0/11.2 \pm 1.5$, high LFSF). Functionality of each of the flours was compared with the functionality of a commercial defatted soy flour (DFSF) by Heywood *et al.* (9). Functionality tests included solubility, emulsification capacity (EC), emulsification activity index (EAI), emulsion stability index (ESI), foaming capacity (FC), foam stability (FS), water-holding capacity (WHC), and fat-binding capacity (FBC).

Protein solubility curves for different E-E flours are compared in Figure 9.6. All three LFSFs and the DFSF had minimum solubility at pH 4.0 and the solubility increased with more basic or more acidic pH, and those receiving more heat treatment had modestly less protein solubility than those receiving less heat treatment. Protein solubility is considered to be one of the most important measures of functionality, because it is an indicator of how the protein will perform in other functionality tests (11). The ECs of the E-E flours are shown in Figure 9.7. EC increased with increas-

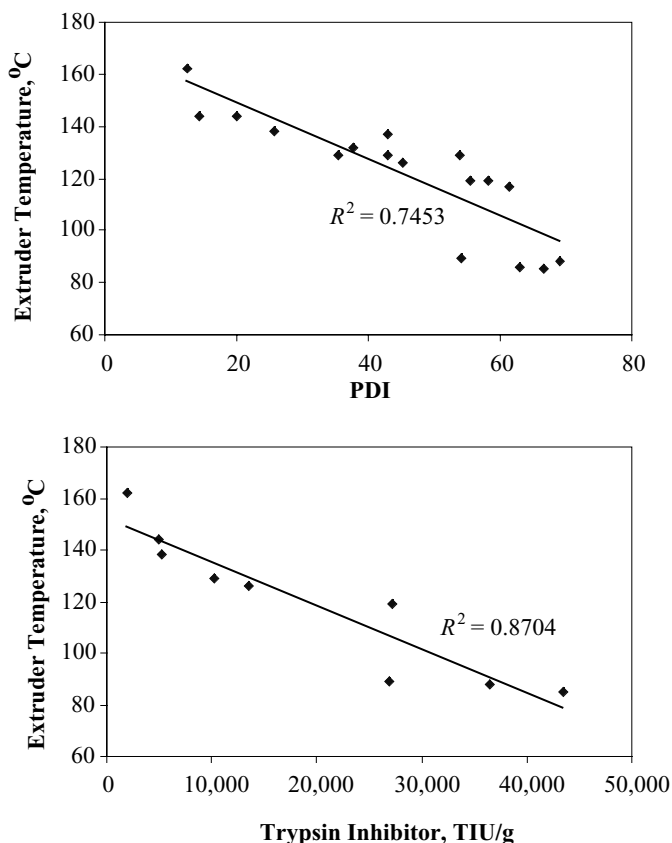


Figure 9.5. Relationship between extruder temperature and denaturation of soy protein and trypsin inhibitor (8).

ing pH and PDI/RO. As the pH approaches the protein's isoelectric point, pI, net electrical charge decreases, reducing solubility and functionality. This was more obvious for the more heat-denatured protein flours.

EAI is a measure of the interfacial area that is stabilized per unit weight of protein. ESI is a measure of the resistance of an emulsion to breakdown. EAI has been found to be highest for low LFSF and lowest for DFSF (Table 9.4). The ESI follows the same trend as EAI. EAI directly relates to oil globule size, and therefore, low LFSF may have resulted in the smallest oil globule size, resulting in the greatest ESI.

WHC was significantly lower for the high LFSF compared with the other samples. This result was attributed to the large amount of RO present in high LFSF.

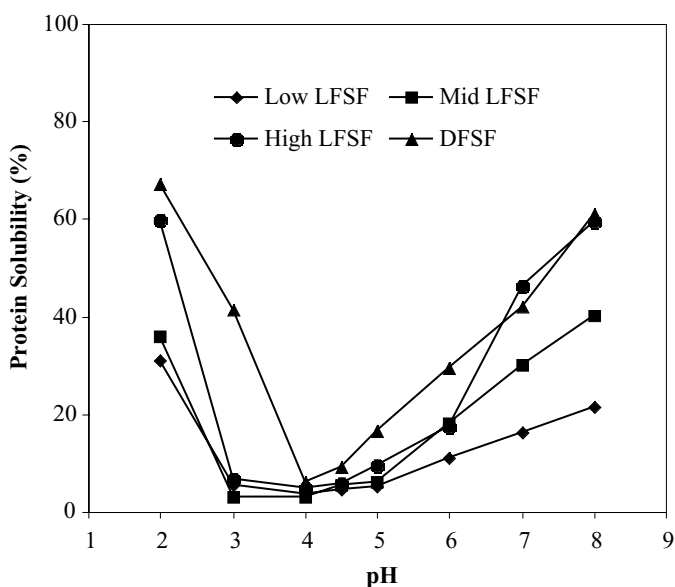


Figure 9.6. Protein solubility curves for low-fat soy flours (LFSF) and defatted soy flour (DFSF) (9).

TABLE 9.4

Functional Properties of Various Soy Flours (9)^a

Treatment	EAI ^b	ESI ^c	WHC ^d	FBC ^e	FC ^f	FS ^g
Low LFSF	15.4 b	12.78 a	6.75 a	1.66 b	0.81 c	0.37 a
Mid LFSF	12.1 a	11.35 b	6.19 a	1.74 b	0.85 a	0.14 b
High LFSF	11.2 a	10.28 c	4.79 b	1.84 b	0.88 b	0.11 c
DFSF	10.8 a	10.36 bc	6.70 a	2.22 a	0.85 a	0.01 d

^aValues followed by same letter in the same column are not significantly different at 95% confidence level.

^bEmulsification activity index, in m^2g^{-1} .

^cEmulsion stability index, in min.

^dWater-holding capacity, g water/g protein.

^eFat-binding capacity, g oil/g protein.

^fFoaming capacity, mL foam/mL $\text{N}^2 \times \text{min}$.

^gFoam stability, $\text{mL}^{-1} \times \text{min}^{-1}$.

DFSF had much higher fat-binding capacity than the LFSF. Residual oil that was present in LFSF may have blocked the hydrophobic binding sites usually available for binding added fat.

FC is a measure of the maximum volume of foam generated by a protein solution, while FS is a measure of the resistance of the foam to destabilization and collapse. The lower the value, the more stable the foam. DFSF and LFSF had significantly different

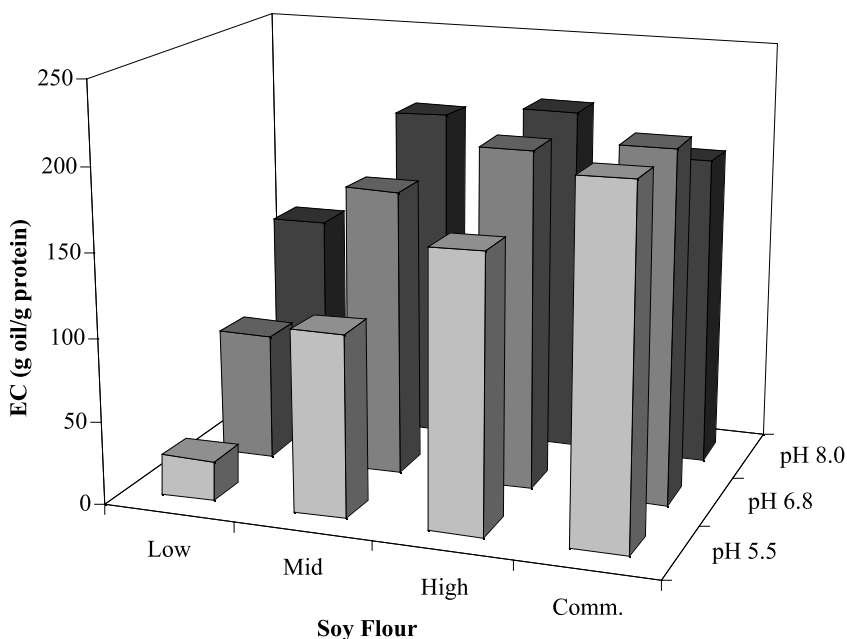


Figure 9.7. Emulsification capacity (EC) of various types of LFSF (Low, Mid, and High PDI/RO) compared with DFSF (commercial defatted soy flour, designated as Comm.) at different pH conditions (8).

foam stabilities. DFSF produced very stable foams, with symmetrical, evenly distributed foam bubbles. As with WHC and FBC capacity, foaming properties of LFSF may be dependent not only on the PDI of the flour but also on RO content.

Functionalities of E-E Flours Produced from Value-Enhanced Soybeans

Heywood *et al.* (12) also studied the functional properties (protein solubility, emulsification characteristics, foaming characteristics WHC, and FBC) of the E-E soy flours produced from six varieties of value-enhanced soybeans. These soybeans included high-sucrose or low-stachyose (LSt), high-cysteine (Hc), low-linolenic (LLL), low-saturated-fatty-acids (Ls), high-oleic (Ho), lipoxxygenase-null (LOX), and two commodity soybeans (Wc and St).

The soy flours varied in PDI (32.0–49.5) and RO content (7.0–11.7%). As expected, there were no significant differences for WHC, FBC, emulsification activity, or emulsification stability among E-E flours prepared from different types of beans. However, the flour characteristics or oxidative stability of these protein products may be different. In general, the PDI and RO values of E-E soy flours had greater influence on protein functionality than seed type did.

Applications of E-E Soy Meal or Flour

E-E Flour in Doughnuts

Defatted soy flour has been used in commercial doughnut mixes (13). The primary purpose of adding soy flour is to decrease the amount of oil absorbed by doughnuts during frying (14). Soy flour also improves gas retention and controls crust color and volume (15). Typical usage level of soy flour in commercial doughnut mix ranges from 1 to 3% of the total wheat flour in the formulation (16). However, there have been studies of the potential of using larger amounts of soy flour to reduce costs (17,18). Most of these efforts involved DFSF in standard cake doughnuts.

Effects of LFSF incorporation on compositional, physical, and sensory attributes of standard cake doughnuts were investigated (Heywood *et al.*, unpublished data). Low, mid, and high PDI/RO (18.2/6.5, 44.9/7.1, and 67.8/11.8, respectively) were compared with a commercially available DFSF (PDI/RO 73.0/0.6). These soy flours were added to the doughnut formulation at 3, 5, and 8% (wheat flour weight basis). LFSF maintained quality and sensory characteristics when added to standard cake doughnuts. However, LFSF did not behave as consistently and predictably as DFSF did. Furthermore, LFSF was not as effective in reducing fat absorption as was DFSF. Sensory panels found that type of flour and addition level both play integral roles in their responses for oiliness, darkness, tenderness, and moistness.

Texturized Soy Protein (TSP) Production from E-E Flour

Extruders are used to produce meat analogs or extenders from plant proteins. TSP is produced primarily by extruding defatted soy flour, soy protein concentrate, and occasionally, soy protein isolate. The exposure of proteins to high temperature, pressure, and mechanical shear in the extruder causes proteins to align parallel to the extruder barrel, and expand when forced through the die. The sudden pressure decrease as the extrudate leaves the die causes water to flash off as steam, resulting in an expanded, porous structure. Riaz's research group at Texas A&M University produced TSP using partially defatted E-E products (19). E-E meal was adjusted to 21% moisture content, and extruded shreds or chunks were obtained by a secondary extruder. These products hydrated readily, resembled ground or chunk meat, and retained a chewy texture when cooked. It was found that an E-E protein product with PDI as low as 25 could be satisfactorily texturized.

Crowe and Johnson (20) studied the effects of PDI and RO content of E-E soy flour on texturizing soy protein and on functionality of the resulting TSP products. Ten partially defatted soy flours with RO contents and PDI values ranging from 5.5 to 12.7% and 35.3 to 69.1, respectively, were texturized by using a twin-screw extruder. The TSP products, including a commercial sample (from Archer Daniels Midland), were tested for WHC and texture of the hydrated TSP. TSP-extended ground beef was evaluated for its sensory quality.

WHCs, bulk densities, and sensory quality of TSS produced from partially defatted soy flour were evaluated. RO content tended to negatively correlate with WHC. WHC negatively correlated with bulk density. Similarly, Rhee *et al.* (21) reported an inverse relationship between WHC and bulk density in extrudates produced from flours with a wide range of nitrogen solubilities. The lack of available water-binding sites made these low-solubility or insoluble protein aggregates unable to incorporate sufficient water to develop proper dough consistency within the extruder barrel. Upon release from the die, the extrudate did not properly expand due to insufficient entrapped moisture as evidenced by decreased bulk density. The bulk density range of partially defatted soy flour extrudates was 0.22–0.26 g/cm³.

Hardness of the TSP was significantly reduced in high-RO samples. The negative correlation between RO and all instrumental texture measurements indicated that the higher lipid contents of these samples may inhibit protein interactions responsible for desirable extrudate textural attributes. Both Faubion and Hosney (22) and Bhattacharya and Hanna (23) found that removing lipids from flours favorably influenced TSP textural qualities, and Kearns *et al.* (24) reported a maximum recommended fat level of 6.5% in raw materials. However, neither PDI value nor RO content affected textural attributes measured in the TSP-extended ground beef system.

Sensory evaluation of TSP-extended ground beef patties indicated that there were no significant differences in hardness or chewiness in the TSP-extended ground beef compared with the control. RO content of partially defatted soy flour strongly correlated with overall flavor. In general, TSP from low-fat, partially defatted soy flour had less soy flavor and better overall flavor compared with TSP from high-fat, partially defatted soy flour.

TSP from Genetically Enhanced Soybeans and Application as Meat Extender

TSP made from soy flours (as described in the previous section, with PDI and RO values ranging from 32.0 to 49.5 and from 7.0 to 11.7%, respectively) of six different varieties of value-enhanced soybeans and two varieties of commodity soybeans were incorporated at the 30% level (rehydrated) into all-beef patties by Heywood *et al.* (25). The value-enhanced varieties included Hc, LLL, LOX, LSt, Ls, and Ho; the two commodity soybeans were Wc and St.

The bulk densities and WHC of the TSPs made with different value-enhanced soybeans were negatively correlated ($r = -0.68$). Moisture content of cooked beef patties ranged from 51.6 to 55.0%, well within the range of other published cooked moisture values (26). Fat levels of all patties varied little, ranging from 16.5 to 17.9%. Protein contents of the cooked patties were also very consistent, with little deviation from 21%.

Cooking parameters (moisture retention, fat retention, cooking yields) and selected texture attributes were also examined. Texture profile analysis showed that the addition of TSP increased hardness of the ground beef patty. TSP-extended beef patties had lower springiness values compared with those of the all-beef control. For

sensory evaluation, panelists detected more soy flavor in all TSP-extended patties compared with the control. However, soy flavor did not deviate significantly between varieties. Finally, chewiness and juiciness scores were not significantly different among TSP-extended patties and the control. Even though instrumental analyses demonstrated some differences between TSP-extended patties and the all-beef control, human subjects did not detect significant difference.

E-E Meal Used as Animal Feed

The majority of E-E meal is currently incorporated into livestock feeds. There are different quality requirements when the protein meal is fed to ruminant animals than when it is fed to non-ruminant animals. Antinutritional factors are of primary concern for non-ruminant animals, whereas the rumen-bypass protein content is the most important quality indicator for ruminant animals.

Compared with SE soy meal, E-E meal has higher oil content and thus contains more energy. Woodworth *et al.* (27) studied amino acid digestibility and digestible energy (DE) and metabolizable energy (ME) of E-E and SE meals when fed to swine. The apparent ileal digestibility of crude protein, lysine, valine, isoleucine, and other amino acids were greater ($P < 0.05$) for the E-E product compared with the SE protein meal. Energy values had the same trend. The SE meal had lower DE and ME compared with those of E-E products. The nutrient compositions of the two products were similar on an equal dry-matter basis. There may be lower nutrient concentration in the animal waste when using E-E meal due to its higher digestibility. A similar study of starter pig feeding examined the effect of type of soybean meal on growth performance (28). Pigs fed with E-E protein diet performed similarly to those fed SE soybean meal with added oil; therefore, E-E meal can replace the conventional product without affecting growth performance.

For lactating dairy cows, soybean meals from different processing methods have different feed performances due to their differences in rumen-bypass or undigestible protein content. Although SE soybean meal has a favorable amino acid profile and high post-rumen protein digestibility, its rumen digestibility is high; thus, less protein passes through the rumen, and less is utilized by the cows (29). Heat treatment, such as roasting and extruding, reduces rumen protein degradation, thus increasing rumen-bypass protein. Socha (30) showed that cows fed extruded soybeans produced 6.6 lb/cow/day more milk than cows fed untreated SE meal or raw soybeans. The quality survey conducted by Wang and Johnson (4) indicated that on average, SE and E-E meals had similar rumen-bypass protein.

References

1. Said, N.W., Dry Extrusion-Mechanical Expelling of Oil from Seeds—A Community-Based Process, *INFORM* 9:139–144 (1998).
2. Nelson, A.I., W.B. Wijeratne, S.W. Yeh, T.M. Wei, and L.S. Wei, Dry Extrusion as an Aid to Mechanical Expelling of Oil from Soybeans, *J. Am. Oil Chem. Soc.* 64:1341–1347 (1987).

3. Bargale P.C., R.J. Ford, F.W. Sosulski, D. Wulfsohn, and J. Irudayaraj, Mechanical Oil Expression from Extruded Soybean Samples, *J. Am. Oil Chem. Soc.* 76:223–229 (1999).
4. Wang, T., and L.A. Johnson, Survey of Soybean Oil and Meal Qualities Produced by Different Processes, *J. Am. Oil Chem. Soc.* 78:311–318 (2001).
5. Herold, D., T. Klopfenstein, and M. Klemesrud, Evaluation of Animal Byproducts for Escape Protein Supplementation, *Nebraska Beef Cattle Report MP 66-A*:26–28 (1996).
6. Araba, M., and N.M. Dale, Evaluation of Protein Solubility as an Indicator of Over Processing of Soybean Meal, *Food Tech.* 69:76–83 (1990).
7. Baize, J.C., Results of USB Study on SBM Quality Released, *Soybean Meal INFOsource*, 1(4):1, 4 (1997).
8. Crowe, T.W., L.A. Johnson, and T. Wang, Characterization of Extruded-Expelled Soybean Meals and Edible Flours, *J. Am. Oil Chem. Soc.* 78:775–779 (2001).
9. Heywood, A.A., D.J. Myers, T.B. Bailey, and L.A. Johnson, Functional Properties of Low-Fat Soybean Flour Produced by an Extrusion-Expelling System, *J. Am. Oil Chem. Soc.* 79:1249–1253 (2002a).
10. Guzman, G.J., P.A. Murphy, and L.A. Johnson, Properties of Soybean-Corn Mixtures Processed by Low-Cost Extrusion, *J. Food Sci.* 54:1590–1593 (1989).
11. Kinsella, J.E, Functional Properties of Proteins in Foods: A Survey, *Crit. Rev. Food Sci. Nutr.* 7:219–280 (1976).
12. Heywood, A.A., D.J. Myers, T.B. Bailey, and L.A. Johnson, Functional Properties of Extruded-Expelled Soybean Flours from Value-Enhanced Soybeans, *J. Am. Oil Chem. Soc.* 79:699–702 (2002b).
13. Martin, M.L., and A.B. Davis, Effect of Soybean Flour on Fat Absorption by Cake Doughnuts, *Cereal Chem.* 63:252–255 (1986).
14. Spink, P.S., M.E. Zabik, and M.A. Uebersax, Dry-Roasted Air-Classified Edible Bean Protein Flour Used in Cake Doughnuts, *Cereal Chem.* 61:251–254 (1984).
15. Gorton, L., Cake Doughnuts Made from Mixes, *Bakers Dig.* 58:8 (1984).
16. French, F., Bakery Uses of Soy Products, *Bakers Dig.* 51:98–103 (1971).
17. Murphy-Hanson, L.A., *The Utilization of Spray Dried Soymilk and Soybean Flour for the Reduction of Fat Absorption during Deep Fat Frying of Cake Doughnuts*, Thesis, Iowa State University, Ames, 1992.
18. Low, Y.C., *The Physical, Chemical and Sensory Properties of Soymilk, Tofu and Doughnuts Made from Specialty Full-Fat Soy Flours*, Thesis, Iowa State University, Ames, 1997.
19. Riaz, M.N., Extrusion-Expelling of Soybeans for Texturized Soy Protein, in *Proceedings of the World Conference on Oilseed Processing and Utilization*, edited by R.F. Wilson, AOCS Press, Champaign, Illinois, 2001, pp. 171–175.
20. Crowe, T.W., and L.A. Johnson, Twin-Screw Texturization of Extruded-Expelled Soybean Flours, *J. Am. Oil Chem. Soc.* 78:781–786 (2001).
21. Rhee, K.C., C.K. Kuo, and E.W. Lusas, Texturization, in *Protein Functionality in Foods*, edited by J.P. Cherry, ACS Symposium Series, American Chemical Society, Washington, D.C., 1981, pp. 51–87.
22. Faubion, J.M., and R.C. Hoseney, High-Temperature Short-Time Extrusion Cooking of Wheat Starch and Flour. I. Effect of Moisture and Flour Type on Extrudate Properties, *Cereal Chem.* 59:529–533 (1982).
23. Bhattacharya, M., and M.A. Hanna, Effect of Lipids on the Properties of Extruded Products, *J. Food Sci.* 53:1230–1231 (1988).

24. Kearns, J.P., G.J. Rokey, and G.R. Huber, Extrusion of Texturized Proteins, in *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, AOCS Press, Champaign, Illinois, 1988, pp. 353–362.
25. Heywood, A.A., D.J. Myers, T.B. Bailey, and L.A. Johnson, Effect of Value-Enhanced Texturized Soy Protein on the Sensory and Cooking Properties of Beef Patties, *J. Am. Oil Chem. Soc.* 79:703–707 (2002c).
26. Anderson, R.H., and K.D. Lind, Retention of Water and Fat in Cooked Patties of Beef and of Beef Extended with Textured Vegetable Protein, *Food Tech.* 29:44–45 (1975).
27. Woodworth, J.C., M.D. Tokach, R.D. Goodband, J.L. Nelssen, P.R. O'Quinn, and D.A. Knabe, Apparent Ileal Digestibility of Amino Acids and Digestible and Metabolizable Energy Values for Conventional Soybean Meal or Dry Extruded-Expelled Soybean Meal for Swine, Preliminary Progress Report presented at Insta-Pro International's Extrusion-Expelling Workshop, Des Moines, Iowa, August 26–27, 1998.
28. Woodworth, J.C., M.D. Tokach, J.L. Nelssen, R.D. Goodband, and R.E. Musser, Evaluation of Different Soybean Meal Processing Techniques on Growth Performance of Pigs, Preliminary Progress Report presented at Insta-Pro International's Extrusion-Expelling Workshop, Des Moines, Iowa, August 26–27, 1998.
29. Shaver, R., How to Evaluate Beans, *Feed Manage.* 50:15–18 (1999).
30. Socha, M., Effect of Heat Processed Whole Soybeans on Milk Production, Milk Composition, and Milk Fatty Acid Profiles, Thesis, University of Wisconsin, Madison, 1991.

Chapter 10

Soy Molasses: Processing and Utilization as a Functional Food

Daniel Chajuss

Hayes General Technology Co. Ltd., Misgav Dov, Emek Sorek 76867, Israel

“Soy molasses” or “soybean molasses” is a brown viscous syrup with a characteristic bittersweet flavor. Soy molasses is a concentrated, desolventized, aqueous alcohol extract of defatted soybean flakes, a by-product of “traditional” aqueous alcohol soy protein concentrate production. “Soy molasses” is a terminology given by Hayes Ltd., which first commercially produced and marketed it in 1963. Soy molasses was thus named to distinguish this then-new aqueous alcohol desolventized soybean extract from “soybean whey” or “condensed soybean solubles,” the by-products of the soy protein isolate and the acid-washed soy protein concentrate production, respectively.

Processing

Soy molasses is manufactured industrially by extracting defatted non-toasted soybean flakes having a nitrogen solubility index (NSI) of 50 to 70, with 60 to 70% warm aqueous ethanol, or when warranted with aqueous isopropanol (IPA); the choice of alcohol extractant depends on the availability and relative prices of ethanol and isopropanol. After extraction, the alcohol and some of the water are removed by such methods as evaporation, distillation, and steam stripping. The end product, soy molasses, is essentially alcohol-free, with desired moisture content (1). It is estimated that more than 100,000 metric tons of soy molasses were produced and available worldwide in 2001.

A modified soy molasses product with reduced soy sugar content is obtainable by partial or complete removal of sugars from the soy molasses. The removal of the sugars present in the soy molasses is accomplished by such methods as microbial fermentation, treatment with various enzymes and chemical hydrolyzing agents, and various physical and chemical procedures, including diverse membrane separation technologies, gel filtration, column separation systems, acid precipitation, and other systems that precipitate the major non-sugar components followed by removal of the soluble components, mainly the sugars, by centrifugation, settling, or decantation (2).

Composition and Utilization

The major constituents of soy molasses are sugars: oligosaccharides (stachyose and raffinose), disaccharides (sucrose), and minor amounts of monosaccharides (fructose and glucose). The composition of soy molasses fluctuates depending on the variety of soybean used, the growing conditions, growing location, and year. Minor constituents include saponins, protein, lipid, minerals (ash), isoflavones, and other organic materials. A typical gross composition of soy molasses is summarized in Table 10.1.

Soy molasses is used as a feed ingredient in mixed feeds, as a pelleting aid, as an addition to soybean meal (e.g., by spraying into the soybean meal desolventizer toaster), mixed with soy hulls, and in liquid feed diets for ruminants (Table 10.2). Pigs are able to digest the oligosaccharides present in soy molasses. The stachyose and raffinose are apparently completely fermented by the hindgut bacteria of the weanling pig (3). Soy molasses can be used as a fermentation aid, as a prebiotic (bifidobacteria growth promoter) (4) and as an ingredient in specialized breads (5). It can be used as a substrate for lactic acid production by *Lactobacillus salivarius* (6), as plywood adhesive (7), and to stabilize sandy loams. Hayes Ltd. sold some appreciable quantities in the late sixties for this last purpose.

The soybean contains various minor constituents mostly held in the past to be deleterious antinutrients. Presently many of these constituents are considered beneficial to treat and ameliorate various pathological conditions. These are labeled “soy phytochemicals” or “soy nutraceuticals.” Soy molasses contains the entire range of soy phytochemicals present in soybeans. Furthermore, soy molasses contains high quantities of soy oligosaccharides as well as varying amounts of soy phytochemicals that may reach five times the amount present in soybeans.

TABLE 10.1
Typical Composition of Soy Molasses on a Dry Matter Basis

Component	Percentage (%)
Soy sugars	58–65
Oligosaccharides	
Stachyose	23–26
Raffinose	4–5
Disaccharides	
Sucrose	26–32
Monosaccharides	
Fructose	1.2–1.6
Glucose	0.9–1.3
Crude protein [$N \times 6.25$] (including amino acids, peptides, etc.)	5–7
Crude lipid material (including phosphatides)	4–7
Minerals (ash)	3–7
Saponins	6–15
Isoflavones	0.8–2.5
Other organic constituents (including phenolic-acids, leucoanthocyanins, etc.) remainder (to 100)	

TABLE 10.2Typical Liquid Feed Formulas^a for Ruminants Based on Soy Molasses

Ingredient	kg/ton	kg/ton
Soy molasses (based on 68% dry solids) ^b	630	630
Water ^b	175	180
Urea	110	95
Urea phosphate	—	45
Phosphoric acid (25%)	35	—
Salt, bentonite	40	40
Vitamins and minerals	10	10

^aCrude protein ~32.00%; metabolized energy (ME) ~1,600 kcal/kg.^bThe amount of water and the amount of soy molasses in the formula are adjusted according to the actual dry matter solids and water provided by the soy molasses.

The major phytochemical components of soybeans are: isoflavones, saponins, phenolic acids, Bowman-Birk proteolytic enzyme inhibitors (BBI), phospholipids and “phytogenic apoptosis inhibitors,” leucoanthocyanins, phytosterols, phytates, omega-3 fatty acids, and likely others not yet ascertained. A lucrative utilization of soy molasses is its use as a source of soy phytochemicals and soy sugars.

There are numerous publications related to the advantageous uses of soy phytochemicals (9–13). Soy molasses and modified soy molasses phytochemical components are considered useful for prevention and amelioration of various pathological conditions such as menopausal syndromes; osteoporosis; hip fractures; hot flashes; breast, colon, lung, prostate, and other types of cancers; prostate hypertrophy; and heart diseases. The Bowman-Birk trypsin and chymotrypsin inhibitor (BBI) along with the isoflavones, saponins, phenolic acids, and other soy phytochemical constituents of soy molasses are considered responsible for the soy phytochemicals’ anticancer properties.

Topical preparations based on soy molasses and/or modified soy molasses can be used to treat dermatological and cosmetic disorders, such as inflammatory pilosebaceous skin diseases characterized by comedones, papules, pustules, inflamed nodules, and superficial pus-filled cysts (acne), and for treatment and amelioration of dermatophyte superficial fungus infections of the skin (athlete’s foot) (8).

Besides the above-mentioned beneficial phytochemicals, soy molasses contains antinutritive factors as noted, for example, in fish diets. Soy molasses had negative effects on nutrient digestibility, growth, and health of salmonids (14,15).

Isoflavones in Soy Molasses

Soy molasses and modified soy molasses are the main raw materials for the production of soy isoflavones. Naim and coworkers in the early seventies first characterized isoflavones in soy molasses (16). They determined biological activities, such as anti-fungal, antihemolytic, and antioxidative activities of the soy isoflavones present in soy molasses. They also discovered a new isoflavone named *glycitein* in soy molasses (16–18). A typical distribution of the isoflavones in soy molasses is given in [Table 10.3](#).

TABLE 10.3

Typical Isoflavone Distribution in Soy Molasses Containing 1.56% Isoflavones (on Dry Solids Basis)^a

Isoflavone Isomer	Percent in natural states
Daidzin	0.23
Genistin	0.36
Glycitin	0.06
Malyl daidzin	0.30
Malyl genistin	0.45
Malyl glycitin	0.04
Acetyl daidzin	0.08
Acetyl genistin	0.02
Acetyl glycitin	0.01
Daidzein	0.01
Genistein	0.01
Glycitein	0.00
TOTAL	1.56

^aHPLC analysis by T. Meredith. The analytical protocol was based on the method of H. Wang and P.A. Murphy, *J. Agric. Food Chem.* 42:1666–1673 (1994).

Barnes *et al.* (19) identified isoflavones and their conjugates in soy molasses and noted that the heat treatment of soy molasses, as in the case of soymilk and tofu, increased the amount of the isoflavone beta-glucosides. Hosny and Rosazza (20) had isolated seven known isoflavones: genistein, daidzein, glycitein, formononetin, genistin, daidzin, and glycitein 7-*O*- β -D-6''-*O*-acetylglucopyranoside, the last a novel isoflavone from soy molasses. Three new isoflavones were also isolated and identified (20).

Most of the publications covering the production of isoflavones from soy molasses come from patent literature. For example: Chaihorsky patented a process for obtaining an isoflavone concentrate from soy molasses by column chromatography (21). Zheng *et al.* (22) patented a process for the isolation and purification of isoflavones from a number of different biomass sources including soy molasses. More specifically, the invention relates to a three-step process whereby a biomass containing isoflavones is immersed in a solvent thereby forming an extract that is subsequently fractionated using a reverse-phase matrix in combination with a step-gradient elution, wherein the resulting fractions eluted from the column contain specific isoflavones that are later crystallized. The purified isoflavone glycosides may then be hydrolyzed to their respective aglycones. For example, genistin was isolated from soy molasses and hydrolyzed to give genistein. Waggle *et al.* (23) disclosed in a patent a series of methods for recovery of isoflavones from soy molasses: (a) a method by which isoflavones are recovered without any significant conversion of isoflavone conjugates to other forms, (b) a method whereby isoflavone conjugates are converted to glycosides while in the soy material prior to their recovery, and (c) a method by which isoflavones are converted to their aglycone form while in the soy material and

prior to their recovery. The extracted isoflavones were separated by HPLC to resolve genistin, genistein, daidzin, glycitin, glycitein, and derivatives thereof. Waggle *et al.* also described various isoflavone-enriched products obtained from soy molasses. Kozak *et al.* (24) disclosed in a patent series the production of a product enriched in isoflavone values from soy molasses by various solvents. Gugger *et al.* (25) patented production of isoflavone fractions from soybeans by using ultrafiltration and liquid chromatography.

A very large and growing body of data is available in the literature on the physiological effects of soy isoflavones. This information is not included herein but may be of interest to readers as most of the commercially available isoflavones are made from soy molasses. A noted example is a study by Setchell *et al.* on the bioavailability of isoflavones and the analysis of commercial soy isoflavone supplements (26). Related valuable information is abstracted and freely accessible at the National Library of Medicine's PubMed web site (27).

Saponins in Soy Molasses

Hosny and Rosazza isolated soysaponin I and soysaponin A2 and a new saponin hexaglycoside IV from soy molasses (20). Berhow, Plewa, and coworkers found that an extract prepared from soy molasses when fractionated into purified chemical components repressed induced genomic DNA damage, whole cell clastogenicity, and point mutation in cultured mammalian cells. A chemical fraction that was isolated from the soy molasses extract using preparative HPLC repressed induced DNA damage in Chinese hamster ovary (CHO) cells. The soy molasses extract was shown to consist of a mixture of group B soyasaponins and 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) soyasaponins. These include soyasaponins I, II, III, IV, V, Be, β g, β a, γ g, and γ a. Purified soyasapogenol B aglycone prepared from the soy molasses fraction demonstrated significant antigenotoxic activity in mammalian cells (28,29).

Other Phytochemicals in Soy Molasses

Phenolic Acids

The following phenolic acids were identified in soy molasses: chlorogenic acid, ferulic acid, gentisic acid, isochlorogenic acid, p-coumaric acid, salicylic acid, syringic acid, vanillic acid, and cinnamic acid (30). Hosny and Rosazza isolated from soy molasses ferulic acid and two cinnamic acid ester glycosides III ($R_3 = OH$, $R_4 = H$; $R_3 = R_4 = OMe$) from soy molasses (20).

Bowman-Birk Inhibitor

The soy molasses contains about 0.2 to 0.5% of the Bowman-Birk trypsin and chymotrypsin inhibitor (BBI). Data in the literature, including patent literature, have shown the ability of crude and purified BBI to prevent or reduce various types of

induced malignant transformations of cells in culture and experimental animals. Kennedy and coworkers provided a review of the literature in one of their U.S. patents (31) as well as more recent data in the previously noted references (10,11) on anticarcinogenesis effects of the Bowman-Birk trypsin and chymotrypsin inhibitor.

Phospholipids and Phytogenic Apoptosis Inhibitors

Wiesner *et al.* (32) isolated a material from soy molasses that is a potent inhibitor in vitro of superoxide anion production in polymorphonuclear leukocytes (PMNs) stimulated with phenol myristate acetate (PMA). This material, prepared by successive extractions with organic solvents, has no protease inhibitory action and was suggested to have possible applications in cancer research and to impart protection against carcinogenesis. This material was later found to be a phospholipid-rich lipid material (33).

Bathurst *et al.* (34) isolated and identified a soybean phospholipid mixture that is a potent inhibitor of apoptotic cell death. This phospholipid mixture was purified from soy fractions, including soy molasses. Analysis of this bioactive lipid mixture identified the two major constituents as phosphatidic acid and phosphatidylinositol. This lipid mixture also contained lesser amounts of lysophosphatidic acid, lysophosphatidylinositol, and lysophosphatidylcholine. These phospholipids had the typical distribution of fatty acids found in soy, predominantly C16:0 and C18:2 (hexadecanoic and 9,12-octadecadienoic) in a 60:40 to 50:50 ratio. Less than 10% of other varieties of fatty acids were identified; the most common other fatty acids found were C18:0, C18:1, and C18:3. Apoptosis inhibition was assessed following serum deprivation of a mouse embryonic stem cell line (C3H-10T1/2). This anti-apoptotic bioassay was used to monitor the purification of the bioactive phospholipid mixture. Of the phospholipids contained in the mixture, lysophosphatidic acid was found to be the most potent inhibitor of apoptotic cell death.

Leucoanthocyanins and Others

An intensely red condensation product with absorbance at 550 m μ was obtained from soy molasses. This leucoanthocyanin-like product is highly unstable and quickly decomposes into colorless derivatives (Chajuss, D., unpublished data). In addition, phytosterols, phytates, and omega-3 fatty acids are soy phytochemicals that are present in soybeans and may also be present in soy molasses, although they are not reported in the literature as being present in soy molasses.

In conclusion, the knowledge about soy molasses composition and its utilization has greatly increased in recent years. Much is still unknown about the very complex mixture of biological constituents termed soy molasses. There is a need for further research. New data on soy molasses and its constituents may yield more information on soy molasses and expand uses of soy molasses and its derivatives as chemoprotective dietetic supplements and for other purposes.

References

1. Chajuss, E.M., and D. Chajuss, Process for the Production of Molasses-like Syrup, Israel Patent 19,186, May 6, 1963.
2. Chajuss, D., Modified Soy Molasses, Israel Patent 119,107, May 9, 1999.
3. Krause, D.O., R.A. Easter, and R.I. Mackie, Fermentation of Stachyose and Raffinose by Hind-gut Bacteria of the Weanling Pig, *Lett. Appl. Microbiol.* 18:349–352 (1994).
4. Hayakawa, K., T. Masai, Y. Yoshida, T. Shibuta, and H. Miyazaki, Enhancing Growth of Bifidobacteria Using Soybean Extract, U.S. Patent 4,902,673, February 20, 1990.
5. Chajuss, D., A Novel Use of Soy Molasses, Israel Patent 115,110, December 8, 1995.
6. Montelongo, J.L., B.M. Chassy, and J.D. McCord, *Lactobacillus salivarius* for Conversion of Soy Molasses into Lactic Acid, *J. Food Sci.* 58:863–866 (1993).
7. Karcher, L.P., The Incorporation of Corn- and Soybean-Based Materials into Plywood, Thesis, University of Illinois, Urbana, 1997. [Abstract, from: *Diss. Abstr. Int.*, 1997B, 57(12), 7297 (1997).]
8. Chajuss, D., Topical Application of Soy Molasses, U.S. Patent 5,871,743, February 16, 1999.
9. Rao, A., and M. Sung, Saponins as Anticarcinogens, *J. Nutr.* 125:771–724 (1995).
10. Messadi, D.V., P. Billings, G. Shklar, and A.R. Kennedy, Inhibition of Oral Carcinogenesis by a Protease Inhibitor, *J. Natl. Cancer Inst.* 76:447–452 (1986).
11. Kennedy, A.R., The Bowman-Birk Inhibitor from Soybeans as an Anticarcinogenic Agent, *Am. J. Clin. Nutr.* 68:1406S–1412S (1998).
12. Thompson, L.U., and L. Zhang, Phytic Acid and Minerals: Effect on Early Markers of Risk for Mammary and Colon Carcinogenesis, *Carcinogenesis* 12:2041–2045 (1991).
13. Newmark, H.L., Plant Phenolics as Inhibitors of Mutational and Precarcinogenic Events, *Can. J. Physiol. Pharmacol.* 65:461–466 (1987).
14. Olli, J.J., Soya i for til laks (*Salmo salar* L.) og regnbueørret (*Oncorhynchus mykiss* Walbaum) [Soybean Products in Diets for Atlantic Salmon (*Salmo salar* L.) and Rainbow Trout (*Oncorhynchus mykiss* Walbaum)], Thesis, Norges Landbrukshogskole, Norway (5719), 1994. [Abstract, from *Diss. Abstr. Int.* 1994C 55/03, 748. (1994).]
15. Krogdahl, A., A.M. Bakke-McKellep, K.H. Roed, and G. Baeverfjord, Feeding Atlantic Salmon (*Salmo salar* L.) Soybean Products: Effects on Disease Resistance (Furunculosis), and Lysozyme and IgM Levels in the Intestinal Mucosa, *Aquaculture Nutr.* 2000:77–84 (1995).
16. Naim, M., B. Gestetner, S. Zilkah, Y. Birk, and A. Bondi, Soybean Isoflavones, Characterization, Determination, and Antifungal Activity, *J. Agric. Food Chem.* 22:806–810 (1974).
17. Naim, M., Isolation, Characterization and Biological Activity of Soybean Isoflavones, Ph.D. Thesis, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel, 1974.
18. Naim, M., B. Gestetner, A. Bondi, and Y. Birk, Antioxidative and Antihemolytic Activities of Soybean Isoflavones, *J. Agric. Food Chem.* 24:1174–1177 (1976).
19. Barnes, S., M. Kirk, and L. Coward, Isoflavones and Their Conjugates in Soy Foods: Extraction Conditions and Analysis by HPLC-Mass Spectrometry, *J. Agric. Food Chem.* 42:2466–2474 (1994).
20. Hosny, M., and J.P.N. Rosazza, Novel Isoflavone, Cinnamic Acid, and Triterpenoid Glycosides in Soybean Molasses, *J. Nat. Prod.* 62:853–858 (1999).

21. Chaihorsky, A., A Process for Obtaining an Isoflavone Concentrate from a Soybean Extract, PCT Int. Patent Application WO9726269, July 24, 1997.
22. Zheng, B., J.A. Yegge, D.T. Bailey, and J.L. Sullivan, Process for the Isolation and Purification of Isoflavone, U.S. Patent 5,679,806, October 21, 1997.
23. Waggle, H., and B.A. Bryan, Recovery of Isoflavones from Soy Molasses, U.S. Patent 6,083,553, July 4, 2000.
24. Kozak, W.G., M.P. Rueter, V. Puvion, J. Patricia, S.I. Kang, and J.D. Thomas, Production of a Product Enriched in Isoflavone Values from Natural Sources, PCT Int. Appl. WO2000032204, 2000.
25. Gugger, E., and R.D. Grabiell, Production of Isoflavone and Fractions by Using Ultrafiltration from Soybeans Liquid Chromatography, U.S. Patent 6,033,714 Cont.-in-part of U.S. 5,792,503, 2000.
26. Setchell, K.D.R., N.M. Brown, P. Desai, L. Zimmer-Nechemias, B.E. Wolfe, W.T. Brashear, A.S. Kirschner, A. Cassidy, and J.E. Heubi, Bioavailability of Pure Isoflavones in Healthy Humans and Analysis of Commercial Soy Isoflavone, *J. Nutr.* 131:1362S–1375S (2001).
27. PubMed Website. Available at www.ncbi.nlm.nih.gov/entrez/query.fcgi. Accessed June 29, 2004.
28. Plewa, M.J., E.D. Wagner, L. Kirchoff, K. Repetny, L.C. Adams, and A.L. Rayburn, The Use of Single Cell Gel Electrophoresis and Flow Cytometry to Identify Antimutagens from Commercial Soybean Byproducts, *Mutat. Res.* 402:211–218 (1998).
29. Berhow, M.A., E.D. Wagner, S.F. Vaughn, and M.J. Plewa, Characterization and Antimutagenic Activity of Soybean Saponins, *Mutat. Res.* 448:11–22 (2000).
30. Chajuss, D., Hayes General Technology Co. Ltd., Unpublished data.
31. Kennedy, A.R., and B.F. Szuhaj, Bowman-Birk Inhibitor Product for Use as an Anticarcinogenesis Agent, U.S. Patent 5,338,547, 1994.
32. Wiesner, R., Y. Birk, D. Chajuss, S. Khalef, S. Smetana, P. Smirnoff, Y. Tencer, and W. Troll, Organic Extractable Materials from Soybeans Inhibit O₂ Production in Stimulated PMNs. Abstract 514 in *Proceedings of Seventy-Fifth Annual Meeting of the American Association for Cancer Research*, Waverly Press, Inc., Baltimore, 1984, p. 130.
33. Birk, Y., Biochemistry and Nutrition Department, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel, Personal Communication.
34. Bathurst, I.C., J.D. Bradley, J.G. Goddard, M.W. Foehr, J.P. Shapiro P.J. Barr, and L.D. Tomei, Soy (*Glycine max*)-Derived Phospholipids Exhibit Potent Anti-apoptotic Activity, *Pharm. Biol.* 36:111–123 (1998).

Chapter 11

Vegetable Soybeans as a Functional Food

Ali Mohamed^a and Rao S. Mentreddy^b

^aVirginia St. University, Petersburg, VA 23806; ^bA & M University, Normal, AL 35762

Hippocrates, 400 BC, said “*Let food be your medicine; medicine be your food.*”

Vegetable soybean, or green vegetable soybean, is one of the traditional soyfoods in many Asian countries (1). In China, it is known as *mau dou*. In Japan, it is known as *edamame* (pronounced “eh-dah-mah-meh”). Basically, vegetable soybean is a large-seeded fresh soybean (*Glycine max* L. Merr.) (seed dry weight > 300 mg/seed) harvested before full maturity when the pods are fully filled and are still green (2). This corresponds to the R₆ growth stage (3). Vegetable soybean has a sweet and delicious taste, and can be eaten as a snack either boiled in water or roasted (4). The fresh beans can also be mixed into salads, stir-fried, or combined with mixed vegetables. In Japan, the beans are ground into a paste with miso, which is then cooked to form a thick broth called *gojiru* (5). *Zunda mochi* in Japan, a popular confectionery vegetable soybean product, is a sticky rice topped with sweetened vegetable soybean paste. Vegetable soybean is also used to make tofu, ice creams, and similar dessert items (6,7). In Asian countries such as China, Japan, Thailand, and Taiwan, vegetable soybean pods are sold fresh on the stem with leaves and roots, or stripped from the stem and packaged fresh or frozen as either pods or beans (Fig. 11.1). In the United States, frozen vegetable soybean products, either in pods or shelled, can be found in markets and are becoming popular as mainstream frozen fresh vegetables (Fig. 11.2). Vegetable soybean is currently gaining popularity with organic growers who target niche commodities for specialty markets and upscale restaurants.



Figure 11.1. Vegetable soybean in the Japanese market.



Figure 11.2. Frozen vegetable soybean in the U.S. market.

The soybean, long prized as an important nutritional component of Asian diets, is now gaining acceptance in Western cultures largely because of its potential health benefits. Soybeans supply all the eight essential amino acids needed for human health. The quality of soy protein is equivalent to that of meat and dairy proteins (8). Soybeans are not only an excellent source of protein, minerals, and vitamins, but are also rich in omega-3 fatty acid, which is associated with the prevention of coronary heart disease in humans (9). More importantly, soybean contains phytochemicals believed to play a role in preventing many chronic illnesses (10). One of the key phytochemical groups identified from soybeans is the isoflavone. This class of phytochemicals has been shown to slow down or prevent the diseases of the heart, prevent certain types of cancers (breast cancer in women and prostate cancer in men), prevent or reduce osteoporosis, and minimize menopausal discomforts among women (11–17). Isoflavones also possess antioxidant and antifungal activity and help plants defend against insects and diseases (18).

Thus, vegetable soybean can be considered as a functional food. Nutraceuticals and functional foods are foods that provide demonstrated physiological benefits or reduce the risk of chronic diseases above and beyond their basic nutritional functions. A functional food is similar to a conventional food, whereas a nutraceutical is isolated from a food and sold in dosage form. In both cases the active components occur naturally in the food. In recent years, the agri-food sector and consumers have begun to look at food not only for basic nutrition, but for health benefits as well. The market for nutraceuticals and functional foods is a large, fast growing, multibillion dollar global industry being driven by a growing consumer understanding of diet and disease links (16,17), aging concerns, rising health care costs, and advances in food technology and nutrition. Governments, the agri-food sector, and the research community are enthusiastic about the potential of vegetable soybean for nutraceuticals and functional foods to improve human health, help growers diversify, and contribute to increased sales of high-value products to niche markets.

As a functional food, vegetable soybean has a strong international market. This chapter addresses vegetable soybean in terms of its brief history, market potential, quality characteristics, nutritional value, phytochemical contents, and agronomic characteristics. Additional information can be found elsewhere (1,2,4,19–22).

Brief History

A comprehensive chronology of vegetable soybean presented by Shurtleff and Lumpkin (1) mentions that although edible soybean in the form of leaf or seeds has first been made in Chinese literature in the seventh century BC, the term *edamame* was used by the Japanese for cooked fresh vegetable soybean pods in 1275 AD (1). The Chinese term *Mao dou*, meaning the “hairy bean,” also called *qingdou*, meaning “green bean,” was mentioned in *Runan Pushi, An Account of the Vegetable Gardens at Runan*, by Zhou Wenhua, published in 1620.

In the United States, the earliest vegetable soybean varieties, introduced from Japan and released by the U.S. Department of Agriculture (USDA), date back to

1915–1916 (23). Approximately 47 varieties were introduced from Asia, most of which were from Japan but 10 came from Korea and 5 from China. Most of these varieties were of maturity groups (MGs) I–IV and a few were MG 0 and VI–VIII. Thus, the Midwest was the targeted region for vegetable soybean production in the United States although no records of acreage of this crop exist (23).

Currently, a few universities in the United States, namely, Iowa State University, University of Illinois at Urbana-Champaign, Washington State University, University of Hawaii, Colorado State University, North Carolina State University, and University of Delaware, have reported limited breeding of vegetable soybean. A few large-seeded soybean varieties particularly suited for vegetable purposes have been released by some of these universities (23–25). Detailed discussion on vegetable soybean breeding is covered in Chapter 14.

Global Market

In the past, vegetable soybean was available only as a fresh vegetable during the harvest season in Japan and many other Asian countries. However, by late 1960s and with improvements in technology, manufacturers began to produce frozen vegetable soybean (7,26,27) that could be made available to consumers yearlong. Japan, China, Korea, and Taiwan have historically been the major producers and consumers of vegetable soybean. Annual production in Japan was 110,000 tons (t) from 1988 to 1992, but production has declined to around 70,000 t today. An additional 70,000 t is imported from other countries (22). By 1974, these same manufacturers also started to expand their production operations overseas to Western countries. Japan continues to be the largest importer of vegetable soybean—fresh or frozen to keep pace with increasing demand.

Taiwan

From just a few hundred tons of frozen vegetable soybean in 1974, Taiwan's production reached a high of 45,000 t per year between 1985 and 1991 (28). By this time, Taiwan had a total of 27 frozen vegetable soybean processors and captured 90% of the Japanese frozen vegetable soybean export market. But with the rising labor and raw material costs in the late 1980s, Taiwanese processors, like Japanese, were forced to expand production operations overseas. As a result, there are only 11 frozen vegetable soybean processors remaining in Taiwan at present (22). These manufacturers export approximately 30,000 t of frozen vegetable soybean per year, of which approximately 24,500 t are exported to Japan, 5,000 t to the United States, and the balance to other countries such as Canada, Europe, and Australia. During the period of the late 1980s, small quantities of fresh vegetable soybean have also been shipped to Japan. However, these shipments have steadily declined because vegetable soybean cannot usually retain freshness by the time it reaches customers. Although China is currently considered the largest frozen vegetable soybean processor, Taiwan will always be regarded as a key supplier of this commodity.

Mainland China

Mainland China opened its doors to foreign investment in the 1980s. Taiwanese processors relocated their operations to southern China because of common language and culture, favorable soil, climate, and close proximity to Taiwan. It took more than seven years to stabilize the vegetable soybean yield (28). The quality of the raw materials has also significantly improved over the years. Currently, China has 10 major Taiwanese companies that operate 16 factories and 30 mainland Chinese owned factories. Together they export about 40,000 t of frozen vegetable soybean to Japan and another 4,500 t to countries including the United States and also Europe and Australia. Due to its relatively cheap labor, China is expected to remain the largest frozen vegetable soybean supplier. However, rising living costs along the coastal areas of China have prompted investors to shift their investments toward the inland rural areas.

Japan

Japan is the world's largest consumer of frozen or fresh vegetable soybean. Frozen vegetable soybean import has increased from 36,200 t in 1986 to 75,000 t in 2000 (28). The Japanese frozen vegetable soybean market is expected to grow further by about 7% per year to 100,000 t by 2005. Although this strong growth is attributable to increasing beer consumption to some extent, particularly among the young Japanese, there are several other reasons as well: First, continuous improvements in frozen-food technology have significantly decreased the peculiar undesirable taste associated with frozen products. As a result, more restaurants, supermarkets, and convenience stores are increasingly replacing fresh vegetable soybean with frozen vegetable soybean. Second, consumers are interested in convenient foods because of fast-paced lifestyles. In response to this trend, Japanese importers and Taiwanese processors produced frozen salted vegetable soybean in the 1990s. Such timely product innovation pushed the demand for frozen vegetable soybean, as demonstrated by the 50,000 t of frozen salted vegetable soybean exported to Japan last year. Third, the aging farming population and decreasing number of young individuals choosing farming as their careers in Japan have led to gradual decrease in fresh vegetable soybean production in Japan every year. Today only 80,000 t of fresh vegetable soybean is consumed against 135,000 t in the 1990s. The demand for frozen vegetable soybean has replaced the demand for fresh vegetable soybean. Finally, the wide variety of vegetable soybean available is expected to spur demand. More than 20 years of research produced new improved vegetable soybean varieties. Recently, *Chamame*, or brown vegetable soybean, and *Kuromame*, or black vegetable soybean, have gained popularity among the Japanese consumers because of their distinctive taste. Interestingly, the darker the color, the more flavorful and sweeter they become. Since their debut three years ago, sales of *Chamame* and *Kuromame* have climbed to 6,000 t per year (28).

Thailand, Indonesia, Vietnam, and Other Countries

In Thailand and Indonesia, production of frozen vegetable soybean began in the 1990s. There are currently three major processors in Thailand. They process a total of 9,000 t per year, of which 8,700 t are exported to Japan and 300 t to the United States and other countries. The quality and the price of Thai frozen vegetable soybean are in between those of Taiwan and China, as reported by Lin (28). Frozen vegetable soybean is expected to grow moderately in Thailand. In 2000, the vegetable soybean production reached about 2,000 t and was exported to Japan. Vietnam had a late start because of its closed-door foreign investment policy. In 1995, it produced 100 t of frozen vegetable soybean. Today, about 250 t are produced. The quality of Vietnam's raw material is still in an early developmental stage. Vegetable soybean is also produced in small quantities in Japan, Australia, and the United States. South American countries such as Argentina and Brazil are aggressively expanding their soybean production and are emerging as major players in international soybean markets. Considering fertile lands combined with abundant cheap labor, these countries could well become major producers of organic vegetable soybean in the future.

The United States

In the United States, vegetable soybean is currently becoming popular and shelled vegetable soybean beans are now available as a frozen fresh vegetable or mixed in stir-fry vegetables (Fig. 11.3) in a few grocery chain stores and oriental food stores. Johnson and colleagues (29) estimated that approximately 13,000 hectare (ha) of vegetable soybean crop is required to meet the demand for fresh or frozen vegetable soybean in the United States. Frozen vegetable soybean imports into the United States increased from approximately 500 t per year in the 1980s to about 10,000 t in 2000 valued at more than \$9 million (28). Taiwan and China are the major suppliers of frozen vegetable soybean to the United States. The vegetable soybean market in the United States is mainly driven by the need for meat alternatives and the growing demand for functional or nutraceutical crops. Thus, it is estimated that by the year 2005, the United States could be importing about 25,000 t of vegetable soybean per

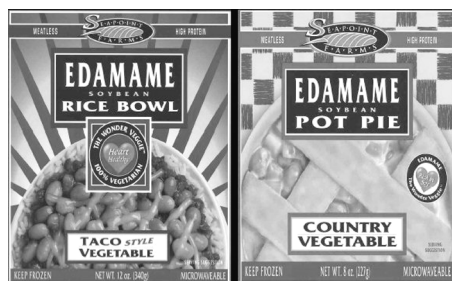


Figure 11.3. Vegetable soybean in processed food.

year (28). In the United States, vegetable soybean is a niche market commodity that fetches a premium price (19). Limited consumer base and lack of suitable cultivars and harvesting machinery are some of the factors limiting vegetable soybean production in the United States. The Asian Vegetable Research and Development Center, Taiwan, responsible for the growing popularity of this crop in the African and Asian countries, has also developed, in addition to releasing several high yielding, good quality vegetable soybean varieties, vegetable soybean cultivars that could be used as a dual-purpose cash and green manure crop, particularly suitable in sustainable organic production systems (7). These varieties produce high pod yields and also a high amount of biomass and, because of their short duration (99 to 120 days for MG V–VII), fit well into existing crop rotation patterns in the southeastern United States.

Quality Characteristics of Marketable Vegetable Soybean

There are a few qualities that are desirable for soybeans to be consumed as vegetables. These include large seed size, soft texture, good flavor, and high amounts of protein, free amino acids, and total sugars (4). Factors affecting these attributes include cultivar, growing seasons, harvest time, and storage conditions. Morphophysical characteristics of the pod and organoleptic properties of the seed determine the marketability of vegetable soybean (7,30).

Chemical Composition and Nutritional Quality of Vegetable Soybean

During seed development and maturation, young soybeans undergo many compositional changes before reaching maturity. During soybean maturation, weight and color change and dry matter increases from 16% to about 90%. However, the average fresh weight of most vegetable soybean varieties, with the exception of a few black-colored varieties such as Tambagura, expressed as mg seed⁻¹, increases from 300 to a peak at 568 and then decreases to about 209 at maturity (20).

Moisture Content. The moisture content of fresh green seeds ranged from 53.9% to 56.1% (31), but the differences between genotypes were not significant. Seed moisture content is another critical factor that affects time of harvest since it is an integral part of organoleptic characteristics of vegetable soybean. Seed moisture content also influences the shelf and storage life of vegetable soybeans. The methods of storage also affect the seed moisture content and, thus, the quality of fresh vegetable soybean (32).

Protein and Oil Accumulations. During maturation, soybeans undergo mass synthesis of storage proteins and lipids. The lipids are stored in oil bodies, mainly in the form of triglycerides, while the proteins are reserved in another organelle known as protein bodies. According to Rubel and colleagues (33), at approximately 25 days

after flowering, the composition of the dry soybean seed is about 30% protein and 5% oil. Yet, this represents only about 70% of the total protein and about 22% of the total oil in the mature seed. From 24 to 40 days after flowering, oil percentage increases rapidly to 13–16% on a dry weight basis, which is about 71% of the total oil in a mature seed (31). At the same time, protein percentage increases to 34%, also representing about 80% of the total protein in a mature seed. During the remainder of the development (about 25 days), dry percentage values of most components remained essentially constant. Since vegetable soybeans are normally harvested between 50 and 60 days after flowering, they contain 11–16% protein and 8–11% oil on a fresh weight basis. In a study conducted in Georgia in the United States (31), the mean protein content of 11 Japanese vegetable soybean genotypes was 36% on a dry weight basis. This is about 86% of the total protein of matured dry bean.

Fatty Acid Composition. Rubel and colleagues (33) found that from 24 to 40 days after flowering the percentage of palmitic, stearic, and linolenic acids in the oil decreases, whereas the percentage of oleic and linoleic acids increases. Although the percent values of the individual fatty acids change markedly, the actual amounts of all fatty acids increase. During the remaining stage of seed development, relative percentages of fatty acids remain essentially constant. However, Sangwan and colleagues (34) and Mohamed and colleagues (35) reported a decrease in oleic acid and an increase in linolenic acid during later stages of seed development (45 days after flowering). The discrepancy among reports might be due to different varieties and assay methods used.

Amino Acid Composition. Of the 17 amino acids detected in soybean seed, arginine, serine, glutamic, glycine, and leucine linearly increase with seed development whereas histidine and alanine linearly decrease, although there was some variation among the two cultivars studied (36). In addition, there is an overall decrease in total free amino acids (37), which may partially explain why vegetable soybeans taste better than mature ones.

Carbohydrates. Sugars detected in soybean seeds include glucose, fructose, galactose, sucrose, raffinose, and stachyose. Sucrose appeared early in the seed development, followed by raffinose and stachyose, which were not detected until 40–50 days after flowering (36). Dimethyl sulfoxide (DMSO) soluble starch reaches a maximum value at 30–40 days after flowering and then declines sharply to almost nonexistent at the mature stage. Vegetable soybeans contain higher amounts of simple sugars and much less in amount of oligosaccharides compared with mature types (36,38). This is consistent with a common impression that flatulence is infrequent after ingestion of vegetable soybeans. The carbohydrate patterns of vegetable soybean are different from those of grain soybean (39). Starch, which is low in grain soybean, makes up 10% of the dry weight of vegetable soybean and the oligosaccharide content of vegetable soybean is very low (40).

Oligosaccharides of soybeans have been generally considered undesirable, because raffinose and stachyose are factors responsible for the flatulence and abdominal discomfort often experienced after ingestion of soybeans. However, these oligosaccharides have been reported to support the growth of bifidobacteria and to play an important role in health benefits from soybean (41). Further details about these compounds are provided in other chapters.

Vitamins. We analyzed a total of 20 vegetable soybean genotypes for tocopherol (42). The three types of tocopherol (δ , γ , and α) and sterols (β -sitosterol, campesterol, and stigmasterol) were measured. Wide variations in tocopherol contents were observed among tested vegetable soybean genotypes. The mean δ , γ , and α -tocopherol contents were 127.6, 84.1, and 97.5 $\mu\text{g/g}^{-1}$ on a dry weight basis, respectively (42). Comparing the growing seasons for 1996 and 1997, there was a significant increase in γ -tocopherol (75 vs. 93 $\mu\text{g/g}^{-1}$) and a significant decrease in α -tocopherol (130 vs. 65 $\mu\text{g/g}^{-1}$). Further information can be found in the literature (43,44).

During maturation, both ascorbic acid and β -carotene decrease and reach their lowest levels at maturity (45). Ascorbic acid in vegetable soybeans could be as high as 40 mg/100 g on a fresh weight basis. It decreases to 2 mg/100 g for soaked weight at full maturity. Similarly, vegetable soybeans contain as much as 0.46 mg/100 g of β -carotene on a fresh weight basis and can be as low as 0.12 mg/100 g for soaked weight when beans are fully matured.

Biologically Active Compounds

Trypsin Inhibitor. On a moisture-free basis, trypsin inhibitor (TI) levels increased with soybean maturation in studied cultivars although there was a difference in the rate of increase (46,47). However, Yao and colleagues (48) observed no changes in TI activities. Thus, cultivar has a great influence in both values and change patterns of TI activities during soybean seed development, but the vegetable soybean generally has lower levels of TIs than mature seeds. Furthermore, TIs in vegetable soybeans are more susceptible to heat destruction than those in mature seeds (38). For vegetable soybeans, boiling in water or steaming for 20 minutes completely eliminated their TI activities (47). However, for mature soybeans, 100% destruction could only be achieved by soaking plus boiling. However, a heating process such as blanching eliminates most of the activities of these inhibitors. One-third of the activity of TI remains in vegetable soybean seed even after boiling for 5 minutes (49).

Phytate. Phytate, a calcium-magnesium-potassium salt of inositol hexaphosphoric acid, commonly known as phytic acid, occurs in certain cereal and legume seeds (50) including soybean (51). Phytate is the main source of phosphorus in soybean seed and is known to form complexes with phosphorus, proteins, and minerals such as Ca, Mg, Zn, and Fe (50). This reduces the bioavailability of these minerals, affects seed germination and seedling growth, and causes deficiencies in nonruminant

animals. Vegetable soybeans also contain a smaller amount of phytic acid, which is widely believed to interfere with mineral absorption in our bodies. The mean phytate content was found to be 1.26% (dry matter basis), with a range of 1.08% to 1.39% in several vegetable soybean genotypes (31). Mebrahtu and colleagues (51) reported slightly higher phytate content for several edible soybeans in Virginia in the United States. Our studies also showed significant variations in phytate among the vegetable-type soybean genotypes as well as between stages of harvests (R_6 and R_7). The significant differences observed for phytate content among genotypes indicated that genetic variation exists among the tested genotypes for selection and improvement through conventional and molecular marker-assisted breeding. According to Liu (47), on a dry matter basis, phytate content increased from 0.84% to 1.36% in one variety and from 0.86% to 1.39% in another during soybean maturation.

Isoflavones. During soybean maturation, there are changes in the total content of isoflavones as well as their isomer compositions (52). In general, malonylgenistin and the genistin contents increased during the latter stages of seed development, whereas malonyldaidzin and daidzin accumulated throughout the whole period (53,54). Minor isoflavone glycosides, such as malonylglycitin and glycitin, were also detected. Isoflavones have been shown to exert many health benefits including cancer prevention and control (55). However, their presence is partially responsible for objectionable taste of soy products (56). Low amounts of isoflavones are consistent with the fact that vegetable soybeans taste less bitter and less astringent than mature types. Isoflavones cause a sour or bitter flavor. Current research indicates that these are important phytochemicals associated with health benefits to humans from soybean. Details are covered in Chapter 3.

Saponins. There is significant variation in saponin content and pattern of accumulation in soybean (57). The variation in saponin composition in soybean seeds is explained by different combinations of five genes controlling the use of soya-sapogenol glycosides as substrate. Phenotypes of more than 1,000 soybeans were classified into eight saponin types, and the frequency of phenotypes was different between the cultivated [*Glycine max* (L.) Merr.] and the wild soybean (*G. soja* Sieb. & Zucc.) (58). The mode of inheritance of saponin types is explained by a combination of codominant, dominant, and recessive genes (58).

A soybean cultivar, Nattoshoryu, contained high amounts (about 6%) of groups B and E saponins in the seed hypocotyl. About 70% of 154 wild soybean (*Glycine soja*) accessions contained arabinosides that are not found in cultivated soybeans, and one accession lacked the group A acetyl saponins. Increased health benefits and decreased undesirable taste (59) in soybeans therefore seems possible through breeding for low levels of desirable saponins (60).

The group A saponins are responsible for an undesirable bitter and astringent taste (53,59,61,62,63). At the same time, however, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-py-4-one (DDMP)-conjugated saponins (64) and their degradation

products, or subgroups B and E saponins (65,66), have health benefits such as inhibition of the infectivity of the acquired immunodeficiency syndrome (AIDS) virus (human immunodeficiency virus or HIV) (67) and inhibition of the activation of the Epstein-Barr virus early antigen (68). The reduction, by genetic means, of saponins possessing undesirable characteristics, together with an increase of the other saponins with health benefits, is important. In this regard, the content of group A saponins is reported to depend more closely on genetic characteristics than on environmental effects (57,60). Therefore, the identification of a group of mutants deficient in group A saponins (57,58,60,69,70) would contribute to the improvement of soybean-based foods

The group A saponins contents (57) and subgroups B and E saponins (60) are not influenced by environmental factors. Because soyasaponin αg is detected only in hypocotyls, soyasaponin αa is detected in cotyledons, and soyasaponin βg is detected in both parts (58,64), it was possible to distinguish among the various seed tissues by analysis of whole seed powders. The data showed no difference among different sowing dates in a report by Tsukamoto and colleagues (53). Therefore, they suggested that, similar to the other saponins tested, DDMP-conjugated saponin contents do not respond to environmental stress in the same manner as isoflavones. Further discussion on soybean saponins is covered in Chapter 4.

Phytosterols. Mean value for β -sitosterol level was found to be $234.8\ \mu\text{g/g}^{-1}$, which was the highest in tested vegetable soybean genotypes, whereas mean levels of campesterol and stigmasterol were significantly lower (45.6 and $44.6\ \mu\text{g/g}^{-1}$, respectively) (71). Comparison by growing seasons showed no significant difference for any of the sterols. These results are in agreement with reported data on mature vegetable and grain-type soybean genotypes (71,72). The concentration of δ -tocopherol in soybean seeds was found to be the highest during the early pod development phase under field conditions, but decreased during the later stages (43). At the same time α - and γ -tocopherols increased.

Given that phytate, tocopherols, phytosterols, and isoflavones have significant health benefits through a reduction in blood serum cholesterol levels, reduction in the risk of cardiac diseases, cancer, and so on, a higher amount of these compounds in vegetable soybean is desirable despite their undesirable effects on organoleptic characteristics.

Nutritional Quality

When compared with corn, green peas, or green beans, vegetable soybeans have four times more fiber and much higher contents of iron, calcium, vitamin C, and protein. The nutritional value and quality of vegetable soybean is superior to that of certain selected soy products such as natto and tofu as well (Table 11.1). Of greater importance is the fact that vegetable soybeans contain higher levels of isoflavones than many other nonsoy food products (4).

Low contents of antinutritional factors and soft texture of vegetable soybeans should improve protein digestibility. Indeed, in one study (73), vegetable soybeans were shown to have higher values of protein efficiency ratio (PER) than mature ones when fed to rats. This pattern is always true whether beans are autoclaved or not. In

another study, the net protein use and PER of vegetable soybeans were found to be comparable to those of casein and lean beef (74).

Organoleptic Features of Vegetable Soybeans

Besides nutritional advantages, vegetable soybeans have several organoleptic features that are superior to mature ones. These include green color, larger seed size, softer texture, sweeter and better taste, and lower beany flavors. Large-seed size results from two factors: high moisture content and genotypic selection for large-seed trait. The age of the seed tissue and genotypic selection account for the soft texture of vegetable beans. The sweet and somehow delicious taste of vegetable soybeans is attributed to their high content amounts of simple sugars and free amino acids and low levels of isoflavones. Quality characteristics and associated chemical compounds are shown in Table 11.2.

Pod and Seed Appearance. Although vegetable soybean is sought for its health benefits, morphophysiological traits determine its marketability and profitability. Pod size, its color, and number of seeds per pod are important morphological traits that

TABLE 11.1
Nutritional Content of Some Vegetable Soybean and Pea Products^a

Composition	Units	Natto	Momen Tofu	Vegetable Soybean	Pea	Green Pea
Energy	Kcal/100 g	200.0	77.0	582.0	30.0	96.0
Water	g/100 g	59.5	86.8	71.1	90.3	75.7
Protein	g/100 g	16.5	6.8	11.4	2.9	7.3
Lipid	g/100 g	10.0	5.0	6.6	0.1	0.2
Nonfibrous						
carbohydrates	g/100 g	9.8	0.8	7.4	5.4	13.0
Fiber	g/100 g	2.3	0	1.9	0.8	2.9
Dietary fiber	g/100 g	—	—	15.6	—	6.3
Ash	g/100 g	1.9	0.6	1.6	0.5	0.9
Calcium	mg/100 g	90.0	120.0	70.0	55.0	28.0
Phosphorus	mg/100 g	190.0	85.0	140.0	60.0	70.0
Iron	mg/100 g	3.3	1.4	1.7	0.8	1.9
Sodium	mg/100 g	2.0	3.0	1.0	1.0	3.0
Potassium	mg/100 g	660.0	85.0	140.0	60.0	70.0
Carotene	mg/100 g	0.0	0.0	100.0	620.0	360.0
Vitamin B ₁	mg/100 g	0.07	0.07	0.27	0.12	0.25
Vitamin B ₂	mg/100 g	0.56	0.03	0.14	0.10	0.12
Niacin						
(mg/100 g)	mg/100 g	1.1	0.1	1.0	0.6	1.9
Ascorbic acid						
(mg/100 g)	mg/100 g	0.0	0.0	27.0	34.0	18.0

^aAdapted from Shanmugasundaram and colleagues (2) and Mbuvi and Litchfield (75).

determine marketability and price of vegetable soybean (2). Generally, pods with more than two seeds in each secure higher prices than those with fewer seeds (2). The pod color is important and bright green is most desirable. Yellowing of the pods reflects declining freshness and degradation of ascorbic acid. Quality properties such as color, texture, and seed size of vegetable soybean are a function of development time (30,37,39,76). Since these quality parameters do not peak at the same time, it is necessary to compromise time of harvest of vegetable soybeans. Shanmugasundaram and colleagues (2) reported that the optimum time for harvesting green beans was when the pods are still green and tight with fully developed green seeds. This stage coincides with the R_6 stage of soybean development (3). Pods bright green in color with gray pubescence and approximately 5.0 cm in length and 1.4 cm in width with two or more bright green seeds having light buff or gray hila are considered important for securing high prices in the Japanese market (2). The color of pods changes from green (R_6) to yellow (R_7) and then to brown or black at maturity (R_8).

The special grade of vegetable soybean should have 90% or more pods containing two or three seeds (30). The pods should be perfectly shaped, completely green, no injuries, and no spots. The grade B vegetable soybean should have 90% or more pods with two or three seeds, but it can be a lighter green, slightly spotted, injured, or malformed, and have short pods or small seeds. The grade A is the intermediate between special grade and grade B. In these three grades, pods must not be overly mature, diseased, insect damaged, one-seeded, malformed, yellowed, split, spotted, or unripe.

TABLE 11.2

Quality Characteristics and Associated Chemical Compounds of Vegetable Soybeans

Characteristic	Associated Chemical Compounds	
Taste	Ascorbic acid, sucrose, glutamic acid, and alanine make green pods and seeds tasty.	
Flavor	<i>cis</i> -Jasmone, and (<i>Z</i>)-3-hexenyl-acetate.	
Nutritional factors	Protein, lipid, fiber, sucrose, ascorbic acid, essential amino acids, vitamins, and minerals	
Antinutritional factors	Phytate	1. Phosphorus, proteins, and minerals. 2. Reduce bioavailability and cause deficiencies of minerals. 3. Significant varietal differences.
	Trypsin inhibitor	Binds proteolytic enzymes and reduces protein efficiency ratio.
	Saponins, isoflavones, and phenolic acids	Sour/bitter and astringent flavor; but associated with health benefits to humans.
	Stachyose and raffinose	Cause flatulence, which leads to abdominal discomfort.

In vegetable soybeans, seed size depends on the genotype and the growing season. Among the yield components, (31,32) seed size varies the most depending upon the growing season, location, and genotype. Seed quality of small-seeded types was superior to the large-seeded types when harvested and tested in reproductive growth stages R_7 , $R_{7.5}$, and R_8 . A simulated weathering treatment using a sprinkler provided a better balance between the biotic and abiotic factors affecting seed quality, including seed compositions (5,39,75). In a study conducted in Georgia in the United States, the mean fresh 100-seed weight of the genotypes tested with the exception of control cultivars varied between 42 and 95 g and the average across 12 genotypes was 51 g (31).

The color of seeds changes from green to light green, yellow-green, yellow, and then to buff-brown. The best time to pick vegetable soybeans for direct consumption is when the seed color changes from green to light green. At this stage, the seeds are at about 80% maturity, sucrose levels are at their peak and many other desirable seed quality traits are also at their peak levels (37).

Texture. Texture also contributes to vegetable soybean quality (77). The soybeans with hard seeds receive low scores. Until the middle pod-filling stage, the seeds tend to have a soft seed coat, which then becomes harder with advancement toward maturity. The vegetable soybean variety Tanbaguro has a large seed size (>950 mg per seed) with moderate texture and strong flavor compared to the seed of many other vegetable soybean varieties (27); therefore, the seed of this variety is highly priced in the Japanese market. Another reason for its premium price is that the seed is considered an important component of many Japanese ceremonies.

The texture of vegetable soybean is rather complex in nature. There is no standard available on the desired texture for vegetable soybean. There are many factors that might contribute to the hardness of vegetable soybean seeds. The hardness of vegetable soybean seeds harvested at different maturity stages is reported by Tsou and Hong (39).

Pods after prolonged cooking are generally softer, and therefore the desired hardness can be obtained through the control of cooking time. Fresh pods are better blanched than boiled to preserve the bright green color and flavor. Pods and seed for the frozen vegetable market are blanched by placing the pods and seeds in boiling water at 100°C for 2–3 minutes, then immersed in cold water at 0°C followed by freezing at -40°C. The blanched pods and seed are stored at -18°C (106). However, extended cooking time may cause the breaking of pods or degradation of pod color.

Flavor and Taste. Rackis and colleagues (78) compared flavor profile in soybeans harvested at different stages of maturity in terms of both beany and bitter flavors. Their taste panel found that flavor intensity values of beany characteristics did not show any significant trends with maturation, but there was a significant increase in

the intensity value for bitter flavor in matured seeds. They attributed the lower bitter flavor partially to lower lipoxygenase activity found in vegetable soybeans; there was an overall increase in lipoxygenase activity in maturing soybeans, although the value fluctuated.

Saponins and isoflavones are responsible for these off-flavors and their thresholds are organoleptically low (79). The higher content of total saponins is observed in the seed hypocotyl fraction than in other seed fractions and ranges from 0.62% to 6.16% (57). The content of saponins in soybean seed varied with the maturity of seed and was more dependent on the variety than on the cultivation year. No information on dry-mouth feeling effects in boiled vegetable soybean is available.

Flavor and texture of boiled vegetable soybean are also highly correlated to their sensory scores. The boiled or blanched soybean contains a characteristic sweet flower-like and beany flavor (2). A combination of ascorbic acid, sucrose, glutamic acid, and alanine make pods and seeds tasty. Whereas *cis*-jasmone, and (Z)-3-hexenyl-acetate have been reported to confer desirable flavor (80,81).

There are many taste-related substances in soybean seed, such as sugars, amino acids, organic acids, inorganic salts, flavonoids, and saponins. Preliminary results show that younger panelists prefer higher sucrose types of vegetable soybean rather than common sweet ones (5,82). Storage experiments of vegetable soybean pod at room temperature showed that sensory panelists could perceive quality differences in freshly harvested soybeans and those harvested 10 hours earlier (27). With the significant increase in vegetable consumption in Western countries and the United States, further studies are required to clarify the contribution of minor components to organoleptic quality. Tsou and Hong (39) indicated that sucrose, which is the predominant sugar in vegetable soybean, is responsible for its sweetness. Therefore, analysis of sucrose content is most important in the evaluation of the sweetness of vegetable soybean (27).

Volatile flavor of the boiled vegetable soybean is highly correlated with quality (30). Sugawara and colleagues (80) investigated the change in flavor components of seeds during the pod-filling stage. The gas-liquid chromatographic (GLC) and GLC-mass spectrometric (GLC-MS) analysis of substances steam-distilled and ether-extracted indicated remarkable differences between vegetable and mature soybean. Characteristic flower-like flavor components of boiled vegetable soybean are *cis*-jasmone, (Z)-3-hexenyl-acetate, linalool, and acetophenone. Major components, 1-octen-3-ol, 1-hexanol, hexanal, 1-pentanol, (E)-3-hexen-1-ol, 2-heptanone, and 2-pentylfuran, the beany flavor (81), are also detected in vegetable soybean (80). Boiling gives seeds their characteristic flavor because of heat-induced substances such as furans and ketones, and easy evaporation of volatiles due to rupture of tissue and cells. Cell rupture accompanied by freezing gives undesirable flavor because of lipid peroxides. Popcorn or pandan-like flavor is perceived in Dedachame or Cha-kaori types. The flavor components might be cyclo N-O substances, eluted by GLC analysis (30).

Factors Affecting Quality Attributes

Harvest time affects vegetable soybean soybean quality mainly because of compositional changes during maturation as discussed earlier. In one report with three veg-

etable soybean cultivars (37), ascorbic acid, sugars, and free amino acids decreased with seed maturation.

Quality improvement of vegetable soybean covers both pre- and postharvest considerations. Seed maturity, growth environment, and cultural practices affect the quality of soybean seeds at harvest. Studies covering pre- and postharvest procedures made it possible to retain freshness, sucrose, and free amino acid levels. Some studies related to genetic control of sucrose and free amino acid levels in legumes, which may lead to quality improvement of vegetable soybean in the future, have been reported. Physiological approaches to controlling sucrose and free amino acids are now being studied in soybean seeds. Research aimed at identification of off-flavor-causing phytochemicals and improving flavor by either eliminating the causative factors through conventional or molecular marker-assisted breeding or through improved processing and storage procedures is in progress in Asia (83).

Genotypes. Over the years, in the Asian countries, vegetable soybean cultivars with traits desirable for fresh consumption have been developed through conventional breeding (2,4,31,51,84). These varieties, once referred to as a “garden type of soybeans” by some Westerners, are now known as vegetable soybean (23). The growth habit of these genotypes is similar to conventional grain soybean bred for oil, but they are generally larger in seed size, tender in texture, lower in beany flavor, higher in protein, and lower in oil and yield. The Japanese varieties tend to have larger seeds with greater flavor than the American genotypes. Having been bred for fresh vegetable, most of the vegetable soybean varieties tend to shatter too easily if taken to maturity (31). The vegetable soybean varieties have large coarse leaves and bear more branches than conventional grain soybean. In some varieties, the pods turn yellow more slowly and thus offer a longer window of opportunity for harvesting tender pods for immediate consumption whereas in some of the varieties the pods tend to quickly turn brown and lose marketable qualities. Significant variations in isoflavones among vegetable soybean genotypes have been documented (54,85–87). In studies reported by Rao and colleagues (31), significant differences exist between vegetable soybean genotypes for both morphological and biochemical components.

Growing Location and Season. The photothermal characteristics of a location determine variety selection. Soybean varieties are classified into maturity groups 000, 00, 0, and 1 through X (88), depending upon their temperature and day-length requirements. Those varieties with the lowest number designation (000 to IV) are considered indeterminate and maturity groups V through X are determinate varieties. Early maturity varieties (000 to IV) are adapted to the more northern climatic regions with the maturity designation increasing as you move south toward the equator. Thus, varieties belonging to maturity groups 000 through IV are more suited to regions of the United States nearer to Canada, characterized by shorter summers and lower temperatures than the southern regions. Cultivars belonging to maturity groups IV and V seem to be more adapted to the central Midwestern United States, whereas higher maturity groups such as VI, VII, and VIII tend to be more adapted

to the southern United States. Most of Taiwanese and Japanese varieties are MG V or lower. Location, climatic patterns, and biotic stress cycles are major considerations in the selection of cultivars. For example, planting a lower MG cultivar early in the season in a higher MG cultivar region could sometimes be a better strategy to avoid yield losses due to possible drought stress or insect pressure that coincides with the critical pod-filling phase of the adapted higher MG cultivars. The lower MG cultivar may produce a higher and better quality yield because it avoids drought stress or insect pressure. However, this will not be a major consideration if irrigation is available. Vegetable soybean yields are generally higher under cooler conditions where temperatures do not exceed 27°C during the pod-filling and seed development phases. In Georgia, where the days are longer and temperatures tend to be up to 30°C, the result is higher fresh-pod and seed yields, but dry mature seeds are of poor quality and have a low rate of germination (31) compared to vegetable soybeans grown in cooler climates. Also, the proportion of two- and three-seeded pods tend to be lower in vegetable soybeans grown in the southern United States than in the cooler climates of the western United States (89) or Taiwan (90) or Thailand (91). Higher temperatures during the seed development phase result in poor quality and shriveled and fewer seeds per pod. In the southern United States, the soybean growing season tends to be longer than that in central or western United States and therefore results in higher pod and seed yields (31).

Seasonal differences influence seed quality and phytochemical contents such as isoflavones, tocopherols, phytosterols, and saponins (85,90,92,93). Chen and colleagues (90) compared seeds produced from spring, summer, and autumn seasons in Taiwan. Poorly filled, damaged, and disease- and insect-affected pods in the spring season were 13%, compared to 6% and 4% of those produced in summer and fall seasons, respectively. Varieties with a large seed size harvested in the spring season also have a lower germination percentage than those harvested in the fall. The results of seed germination after storage of seeds harvested from different seasons were different. For example, the germination of seeds harvested in the spring decreased rapidly after five months in storage under ambient room temperature, whereas the seed harvested in the fall maintained more than 85% germination even after one year of storage under the same conditions. The location and crop season characterized by the differences in environmental characters influence the seed weight and germination rate (90). Similar changes were also reported for seed composition (85,92). Large-seeded vegetable soybean varieties were reported to have poor germination (31,94). Published research also showed that with different seed sizes within a variety, small seeds had better germination than larger seeds (90). Under simulated weathering conditions, Horlings and colleagues (76,95) found that germination was negatively correlated with 100-seed weight.

Akazawa and Fukushima (96) reported both genotypic and year-to-year variations in free amino acids, total sugars, proteins, and starch contents of vegetable soybean. The free amino acids were generally higher in vegetable soybean cultivar than in conventional grain soybean cultivar whereas the year-to-year variation depended

on solar radiation from flowering to harvest. Seasonal differences are manifest in variation in solar radiation, temperatures, day length, and precipitation.

Tocopherol metabolism in developing seeds of vegetable soybean was examined to determine whether temperature, drought, or atmospheric CO₂ influenced either the total amount of tocopherols or the relative distribution of the three major forms of tocopherols present in soybean seeds— α -, γ -, and δ -tocopherol (α TC, γ TC, and δ TC) (97). Small increases in temperature caused large increases in α TC, with levels increasing from 5% to 10% of total tocopherols to as much as 50% (97). There were corresponding decreases in the proportion of δ TC, suggesting that metabolic throughput was affected. Under optimal conditions, seeds were evidently able to synthesize large amounts of α TC. Tocopherol metabolism also appears to be influenced by environmental stresses such as drought, indicating that phytonutrients such as vitamin E may be influenced by weather.

Preharvest. Cultural practices of vegetable soybean are similar to those for commercial grain-type soybean. However, to produce high-grade vegetable soybean, better crop management practices must be applied. Problems with insect and cyst nematode should be closely monitored (98). Details of the optimal crop management practices for producing good quality vegetable soybean have been published for Taiwan (2), the West Coast in the United States (99), and the southeastern United States (31,32).

Period of Harvest. The optimum time for harvesting fresh vegetable soybean to combine the best product quality with maximum yield is rather complex and it is often a compromise depending upon the consumer, the market, and the end-product requirements (75). Because the quality is mainly evaluated by the appearance, the superiority or inferiority of production districts is decided by propriety of harvest period and by postharvest processing. It is always difficult to decide the time of harvesting, because the pods are still filling. To determine the most suitable period for harvesting, the relationships of days after flowering, pod expansion, seed components, and pod color have been investigated.

The length and width of pods can be known relatively early during the growth period, and thereafter seeds rapidly expand. The thickness and weight of pods increase after the pod expansion. Taste of the vegetable soybean is highly correlated to the sucrose content or glutamic acid of seed (27). Therefore, the sugar and free amino acid contents provide a good estimate of the tastiness of vegetable soybean. The taste is known to deteriorate in the latter stages of development, mainly due to the decrease in content of sugars and free amino acids.

Pod color is important for evaluation of the grades (100). Harvested pods are graded into four classes, A, B, C, and D, with A being the best pods and D being the pods with the most undesirable traits. The detailed procedures of grading are provided elsewhere (2,101). Vegetable soybean is harvested at about 33–38 days after flowering (DAF) depending on pod color and thickness. The pods at harvest are

generally bright green in color and lose their brightness after harvest. Good qualities of vegetable soybean are good taste, deep green color of pods, full expansion of pods, and uniform pods without infections or injuries. To obtain uniform pods, it is important to protect plants against diseases and insects. The other three factors can be related to the time of harvest. The reported data indicated that free amino acids decreased after pod expansion, so it is better to harvest as early as possible. With regard to sugar content, conflict exists in literature. In one study, total sugar content was low before 35 DAF and achieved a relatively higher level after 35 DAF (101). In another report, sucrose level increased during early developing stages, but 35 DAF the level tended to decline (102). Furthermore, Masuda (30) reported diurnal changes in sucrose and free amino acid levels of seed at 33–36 DAF. Taste is decided not only by the amount of both sugars and free amino acids in the fresh seed, but also by flavor and texture. Using pod color as a guide, it is suitable to harvest before 40 DAF.

The sensory scores of the boiled vegetable soybean, harvested at different times of the day, showed no significant differences in sweetness, texture, and overall scores except for flavor (30). Both harvest time in terms of number of days after planting and harvesting hour in the day affect the quality of vegetable soybean. The data also showed that after harvest, the shorter the time before blanching and cooling, the better the quality. Development of time-saving procedures on a large scale before blanching or cooling is a major concern.

Harvesting. Most vegetable soybeans are harvested by hand. In Taiwan and Thailand where the use of farm labor is relatively more economical than in most Western countries, the pods are harvested fresh, early in the morning from 2 AM through 10 AM (101). The fresh green pods and seeds are known to retain most of the flavor and freshness when harvested before the temperatures rise in the morning. When the vegetable soybeans are sold in markets still attached to the stems, the plants are hand cut or pulled out by the roots, and unacceptable pods and lower leaves are culled, and the branches tied together in small bundles. For the sale of pods alone, plants are cut and the pods are stripped off. After sorting, 300–500 g of pods are put into a polyethylene net bag and 10 or 20 bags are packaged in a corrugated cardboard box.

Because of the significant increase in acreage after increasing demand, Asian Vegetable Research and Development Center (AVRDC) scientists developed machinery to enable mechanical harvesting and postharvest handling of vegetable soybean. Electric-powered, stationary pod strippers and packaging are also available and commonly used (103,104).

Postharvest Handling. Those pods having only one seed or those injured or diseased are removed by hand. This is a costly and time consuming, labor intensive operation. About 70% of the production time for vegetable soybeans is at the postharvest and processing stages, such as harvesting, stripping pods, sorting, and

packaging (30,77). However, because the market value of vegetable soybeans is mainly determined by their appearance, the sorting process is extremely important, and a producing area that excels at processing and sorting is given a superior rating by consumers. There are many sorting standards in each production district.

Research concerning quality degradation is limited. Vegetable soybeans belong to the vegetable group with a high rate of respiration. After harvest, sugar content decreases rapidly at higher temperatures. Free amino acids also decrease in a short period; content of alanine and glutamic acid was reduced to two-thirds and one-half of the harvest, respectively, when the pods were placed under room temperature ($26 \pm 2^{\circ}\text{C}$) and 66% humidity for 24 hours. In this case, a decrease in sweetness and taste could be recognized after 10 hours (27).

The changes in the quality of pods attached to the stem with leaves and roots or of the stripped pods was studied after harvest. Iwata and colleagues (77) reported that pods on the stem possessed better quality than stripped pods, whereas Osodo (105) reported the contrary. Iwata and colleagues (77) reported that the pods maintained bright green color when they were wrapped with a low-density polyethylene film. The pod color deterioration is accelerated under low humidity conditions, whereas deterioration is prevented under high relative humidity. The fresh green seeds packed and sealed in airtight plastic bags could be stored for about a year when placed in controlled environment chambers set at $15\text{--}20^{\circ}\text{C}$ temperature and 50% relative humidity (106). The pods stripped by machine often turned brown after two or three days, because the browning substances such as phenol oxidases are enzymatically synthesized within the injured cells.

Handling soybeans under cool conditions is important to maintain their high quality. Most vegetables are precooled in summer, in two ways: (a) air-cooling and (b) vacuum-cooling. For vegetable soybeans, vacuum-cooling is effective in maintaining their good quality, because the temperature can be reduced quickly. It is important for quality maintenance to save time in harvesting and sorting to the start of precooling. Minamide and Hata (37) reported that after harvest, ascorbic acid and free amino acids in vegetable soybeans decreased rapidly but total sugar content remained almost unchanged during seven-day storage at 20°C . Increases in protein and starch contents with storage were also reported (77,107,108).

Vegetable soybeans are packed in net bags and then put into corrugated cardboard boxes. The following procedures should help maintain the quality of soybeans in high humidity by (a) spreading moisture absorbing sheets in a box, or (b) preventing transpiration by wrapping the soybeans with polypropylene film instead of the net bag. Use of these materials is planned not only for quality maintenance but also to compete with other production areas. The high humidity seems effective in preventing wilting and maintenance of the deep green pod color.

Some reports indicate that vegetable soybean qualities might change during cold storage, for example, loss of moisture, vitamin C, sugar, and amino acid, and chlorophyll degradation (77,108). Proper storage conditions are essential for vegetable soybean to maintain its quality. As indicated by Tsay and Sheu (109), precooling was effective in

maintaining better quality vegetable soybeans during storage. Tsay and colleagues (110) reported that 1°C is the best temperature for vegetable soybean storage. According to the results of Hsieh and Tsay (111), 3°C is the best precooling temperature for vegetable soybeans. Polyethylene (PE) or Polypropylene (PP) bags with 0.32% pores also can maintain good quality of vegetable soybeans (107). The PE bag-packed samples retained more vitamin C, remained greener, and suffered less weight loss than that packed in net bags. The hardness of all samples increased during storage, with the 20°C-stored samples having the highest increase. The 0°C- and 5°C-stored samples had similar profiles, and the samples in net bags became harder than those packed in PE bags with ethylene absorbent materials (109).

Tsay and Sheu (109) reported that after storage for 16 days, the samples stored at 5°C and 20°C in PE bags with ethylene absorbent materials maintained more than 99% and 97% fresh weight. But the cold storage samples of net bags maintained only 80% fresh weight and the samples stored at 20°C lost 70% of their fresh weight. Regardless of storage temperature or bag type, the data indicated that vitamin C content decreased during storage (108). Vegetable soybean stored at 0°C had the lowest changes in color index. However, after storage at 0°C for 24 days, the color index of net bag-packed vegetable soybean was 10 times that of the PE bags packed with ethylene absorbent materials or with ethylene absorbing film.

Effect of Processing. Murphy (112) studied the effect of cooking retail vegetable soybean beans (without pods) or “green soy peas” by boiling or microwave radiation according to the package directions. Cooking in a microwave resulted in a lower loss of isoflavones to cooking in boiling water (112,113). Thus, microwave-heating vegetable soybean in the pods, or for the shelled beans rather than boiling in water, allows for a greater retention of isoflavones. Also, cooking the green pods allows greater retention of isoflavones compared to shelled beans (112).

Murphy (112) and Anderson and Wolf (114) also measured the group B saponins found in soybeans. The saponin levels in the raw vegetable soybean beans are higher than in mature soybeans. Cooking according to package directions by boiling and microwave heating did not result in any statistical differences in saponin levels in shelled beans or green pods in contrast to what we observed with isoflavones. The saponin levels in a variety of other soy foods were comparable to the saponin levels in vegetable soybean. Soy germ, typically not a food source, is a very concentrated source of saponins.

According to Liu (47), during thermal processing, trypsin inhibitors decreased at a much faster rate in vegetable soybeans than mature beans when both types of beans were not presoaked, presumably due to high initial moisture content. There was also a decrease in oligosaccharide upon heating, but phytate showed little change.

Agronomic Performance in the United States

To reduce dependence on imported vegetable soybean, the USDA funded research to study and select vegetable soybean varieties that can be produced under condi-

tions in the United States. Several programs were funded under this initiative (<http://cris.csrees.usda.gov>).

Vegetable soybean is grown much the same way as conventional grain soybean. However, some MG VI and VII Japanese varieties tend to grow large with extensive branching and may require wider spacing than conventional grain soybean. The vegetable soybean seeds are large (mean seed dry weight, 30 to 60 g 100⁻¹) and therefore need to be planted in moist soil (31). In the United States, information on agronomic and nutritional characteristics of vegetable soybean is very limited. The green bean yields from a wide range of vegetable soybean germplasm lines and varieties reported from three or four locations in United States are comparable with yields of vegetable soybean grown in Taiwan, a major vegetable soybean-producing and exporting country. In the United States, MGs I through III have been reported to be suitable for production in Washington, Oregon, Colorado, and Montana (29,103). In the southeastern United States, maturity groups V through VIII have been found to be suitable for production (31). In a study comprising 15 varieties and breeding lines of Asian origin in Washington, the mean marketable yield of fresh pods ranged from 7.3 to 16.0 Metric tons (Mt) ha⁻¹. The varieties belonged to MG III and IV and matured within a mean 111 days of planting. In an earlier study, Konovsky and colleagues (115) evaluated 36 vegetable soybean genotypes (32 Japanese, three U.S., and one Taiwanese) for yield heritability and quality traits in Washington. Gross yields ranging from 11.2 to 13.6 Mt ha⁻¹ and net yields of around 7.2 to 8.4 Mt ha⁻¹ were reported. This compares well to the mean pod yield of 10 to 13, 6 to 9, and 6 to 10 Mt ha⁻¹ from MG V varieties grown in Taiwan during spring, summer, and autumn seasons, respectively. The vegetable soybean improvement program at the Asian Vegetable Research and Development Center has reportedly increased pod yields of some Taiwanese vegetable soybean varieties to about 24 Mt ha⁻¹ (103). In Colorado, Johnson and colleagues (29) reported green bean gross yields ranging from 2.2 to 10.2 Mt/ha⁻¹. In Alabama, the mean yield of three commercial vegetable soybean varieties ranged from 0.25 to 3.3 Mt ha⁻¹ (116). The varieties may have been of MG III or IV and hence the low yields.

In a four-year multiinstitutional regional soybean research project entitled "Improvement of Soybean for Food Uses" sponsored by the Association of Research Directors of 1890 Historically Black Universities and Colleges with funding from USDA/CSREES, the yield potential of several Asian vegetable soybean genotypes were evaluated in Alabama, Georgia, Maryland, and Virginia. In this study, 10 vegetable soybean cultivars and plant introduction of Japanese origin, two cultivars from China, and two U.S. elite soybean cultivars were evaluated for fresh pod and seed yield, and fresh seed nutritional traits. The results of this study from Georgia are discussed in detail elsewhere (31).

In Alabama, three-year average fresh pod and seed yields ranged from 3.8 to 6.4 Mt ha⁻¹ and 2.2 to 4.7 Mt ha⁻¹, respectively, under rain-fed conditions (32). In a five-year study conducted as part of this regional research project, in Georgia (31), the mean fresh pod and seed yields ranged from 15 to 22 Mt ha⁻¹ and 7.3 to 11.6 Mt ha⁻¹, respectively. The mean number of days from planting to the R₆ stage

when fresh pods were harvested ranged from 95 for MG IV varieties to 136 for MG VI and VII plant introductions and varieties in Alabama, and from 75 to 137 in Georgia. This study showed the importance of MG of the variety adapted to a particular region. At both locations, the genotypic variation was significant and the varieties/plant introductions of Japanese origin outyielded those of Chinese and U.S. origin. The green seed yield at the R_6 stage was significantly correlated with number of green pods at both locations and with seeds only at Georgia location. In Georgia, the fresh green seed yield showed a greater correlation with pod yield than with number of pods and seeds, perhaps because pod yield is the product of number of pods and seeds per pod. The fresh green seed weight showed a positive correlation with number of days to R_6 stage at both locations. The longer duration to attain R_6 stage helped seed development resulting in heavier seeds. Thus, Japanese cultivars Tambagura, Shangrao Wan Qingsi, Akiyoshi, and plant introductions 181565 and 200506, which took longer time (124–134 Days After Planting) to attain R_6 stage, also had heavier seeds. The results of the five-year study at the Georgia location are discussed in greater detail by Rao and colleagues (31). The differences in maturity groups appeared to have a greater influence on fresh green pod and seed yields. At both locations, all Japanese cultivars except Mian Yan flowered later and achieved the R_6 stage later than Hutcheson, which belongs to maturity group V. Stepwise regression analysis by using the Georgia location data on yield components, excluding maturity group, indicated that at the R_6 stage, fresh pod weight (product of number of pods and seeds per pod) was the major determinant of yield with an R^2 value of 0.88 followed by number of seeds m^{-2} , 100-seed fresh weight, and seeds per pod in the order of importance.

Constraints and Future Research Needs

Although vegetable soybeans have several nutritional and organoleptic advantages over mature soybeans, at present the market is very limited, mainly because of difficulty in harvesting. Tender vegetable soybeans are very prone to damage or bruise during harvesting. When they are bruised or damaged, oxidative reactions occur rapidly, leading to off-flavor formation and surface browning. Other constraints include a short period of shelf-life, some degree of hard-to-eliminate beany flavor, overall low field yield compared with mature beans, and lack of marketing efforts. In addition, green color limits their use only as vegetable.

To meet the demand for vegetable soybean, more emphasis should be placed on the introduction of new varieties with higher nutritional quality, and higher yield, development and improvement of agricultural practices and technology for the production of organic vegetable, development of better technology for fast-freezing vegetable soybean with an emphasis on packing technology to increase self-life of the vegetable soybean seeds, improved storage condition for frozen and chilled vegetable soybean, and development of marketing strategies to enhance the distribution. Research also should be directed toward food technology and processing of new products from the vegetable soybean.

In summary, as a green vegetable, vegetable soybeans are highly nutritious, as indicated by their high content of seed protein, oil, ascorbic acid, β -carotene, fiber, iron, and calcium, and low levels of trypsin inhibitors, oligosaccharides, and phytate. They have tender texture, sweet and delicious taste, and versatility for processing. They also contain high amounts of isoflavones. Therefore, the outlook for the market of vegetable soybeans appears promising. However, our success for expanding such a market depends largely on our efforts to solve certain constraints associated with production, harvesting, processing, and marketing of vegetable soybeans. Apparently, solution of these problems requires collaborative research work among people with different disciplines, including food scientists, genetists, plant breeders, engineers, and marketing specialists. Current research revealing the health benefits of soyfoods will no doubt serve as a driving force for us to tackle these challenges.

References

1. Shurtleff, W., and T.A. Lumpkin, Chronology of Green Vegetable Soybeans and Vegetable-Type Soybeans, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, WA, 2001, pp. 97–103.
2. Shanmugasundaram, S., S.-T. Cheng, M.-T. Huang, and M.-R. Yan, Varietal Improvement of Vegetable Soybean in Taiwan, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center, Taipei, Taiwan, 1991, pp. 30–42.
3. Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington, Stage of Development Descriptions for Soybeans, *Glycine max* (L.) Merrill, *Crop Sci.* 11:929–931 (1971).
4. Liu, K., Immature Soybeans: Direct Use for Food, *INFORM* 7(11):1217–1223 (1996).
5. Konovsky, J., The Relationship of Consumer Preference to Amino Acid and Sugar Content of Edamame, *Ikushugaku Zasshi (JJ Breeding)* 40:228–229 (1990).
6. Shanmugasundaram, S., The Evolving Global Vegetable Soybean Industry, in *Proceedings of the 2nd International Soybean Processing and Utilization Conference*, edited by A. Duchanan, Bangkok, Thailand, 1999, pp. 472–478.
7. Shanmugasundaram, S., Global Extension and Diversification of Fresh and Frozen Vegetable Soybean, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, WA, 2001, pp. 161–165.
8. Lewandowski, J., The Joy of Soy: How Healthy Is the Ubiquitous Bean? Better Nutrition, January 2003. Available at www.betternutrition.com/. Accessed July 9, 2004.
9. Nair, S.S., D.J.W. Leitch, J. Falconer, and M.L. Garg, Prevention of Cardiac Arrhythmia by Dietary (N-3) Polyunsaturated Fatty Acid and Their Mechanism of Action, *J. Nutr.* 127:383–393 (1997).
10. Kuo, S.M., Dietary Flavonoid and Cancer Prevention: Evidence and Potential Mechanisms (Critical Review), *Oncogenesis* 8(1):47–69 (1997).
11. Walsh, P.C., Risks and Benefits of Soy Phytoestrogens in Cardiovascular Diseases, Cancer, Climacteric Symptoms and Osteoporosis, *J. Urol.* 168(4, Pt. 1):1637 (2002).
12. Chiechi, L.M., G. Secreto, M. D'Amore, M. Fanelli, E. Venturelli, F. Cantatore, *et al.*, Efficacy of a Soy Rich Diet in Preventing Postmenopausal Osteoporosis: The Menfis Randomized Trial, *Maturitas* 42(4):295–300 (2002).

13. Kris-Etherton P.M., K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, *et al.*, Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer, *Am. J. Med.* 113(Suppl. 9B):71–88 (2002).
14. Suthar, A.C., M.M. Banavalikar, and M.K. Biyani, Pharmacological Activities of Genistein, an Isoflavone from Soy (Glycine max): Part II—Anti-cholesterol Activity, Effects on Osteoporosis & Menopausal Symptoms, *Indian J. Exp. Biol.* 39(6):520–525 (2001).
15. Anthony, M.S., T.B. Clarkson, and J.K. Williams, Effects of Soy Isoflavones on Atherosclerosis: Potential Mechanisms, *Am. J. Clin. Nutr.* 68(6, Suppl.):1390S–1393S (1998).
16. Lichtenstein, A.H., Soy Protein, Isoflavones and Cardiovascular Disease Risk, *J. Nutr.* 128:1589–1592 (1998).
17. Lee, H.P., L. Gourley, S.W. Duffy, J. Esteve, and N.E. Day, Dietary Effects on Breast Cancer in Singapore, *Lancet* 337:1197–1200 (1991).
18. Burden, B.J., and D.M. Nerris, Role of the Isoflavones and Coumestrol in the Constitutive Antagonists Properties of “Davis” Soybean Against an Oligophagous Insect, the Mexican Bean Beetle, *J. Chem. Ecol.* 18:1069–1081 (1992).
19. Carter, T.E., Jr., and R.F. Wilson, Soybean Quality for Human Consumption: Soybeans Role in Australia, presented at the Proceedings of the 10th Australian Soybean Conference, Brisbane, Australia, Sept. 15–17, 1998.
20. Liu, K., *Soybeans: Chemistry, Technology, and Utilization*, Kluwer Academic Publishers, New York, 1999, 11 chapters.
21. Lumpkin, T.A. and S. Shanmugasundaram (Eds.), *Proceedings of the Second International Vegetable Soybean Conference*, Washington State University, Pullman, 2001.
22. Shanmugasundaram, S., High Value Vegetable Soybeans from AVRDC—The World Vegetable Center, *INFORM* (2004).
23. Bernard, L.R., Breeding Vegetable Soybeans in the Midwest, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, 2001, p. 21.
24. Shurtleff, W., and A. Aoyagi, *Bibliography of Fresh Green Soybeans*, Soyfoods Center, Lafayette, CA, 1991.
25. Lumpkin, T.A., J.C. Konovsky, K.J. Larson, and D.C. McClary, Potential New Specialty Crops from Asia: Azuki Bean, Green Vegetable Soybean Soybean, and *Astragalus*, in *New Crops*, edited by J. Janick and J.E. Simon, Wiley, New York, 1992, pp. 45–51.
26. Masuda, R., Freezing of Vegetables: Green Vegetable Soybean, *Refrigeration* 64:359–376 (1989).
27. Masuda, R., K. Hasbizume, and K. Kaneko, Effect of Holding Time Before Freezing on the Constituents and the Flavor of Frozen Green Soybeans (Green Vegetable Soybean), *Nihon Shokuhin Kogyo Gakkaishi* 35:763–770 (1988).
28. Lin, C.-C., Frozen Edamame: Global Market Conditions, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, 2001, pp. 93–96.
29. Johnson, D., S. Wang, and A. Suzuki, Edamame: A Vegetable Soybean for Colorado, in *Perspectives on New Crops and New Uses*, edited by J. Janick, ASHS Press, Alexandria, Virginia, 1999, pp. 385–387.
30. Masuda, R., Quality Requirement and Improvement of Vegetable Soybean, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center, Taipei, Taiwan, 1991, pp. 92–102.

31. Rao, M.S.S., A.S. Bhagsari, and A.I. Mohamed, Yield, Protein, and Oil Quality of Soybean Genotypes Selected for Tofu Production, *Plant Foods Hum. Nutr.* 52:241–251 (1998).
32. Ceibert, E., and V.T. Sapra, Cultivar Trials and Agronomic Characteristics, in *Final Report: Improvement of Soybean for Food Uses: A Regional Research Project, 1994–1999*, compiled by E. Ceibert, V.T. Sapra, H.L. Bhardwaj, and H. Dodo, Association of Research Directors, Inc., 2000, pp. 2–9.
33. Rubel, A., R.W. Rinne, and D.T. Canvin, Protein, Oil, and Fatty Acid in Developing Soybean Seeds, *Crop Sci.* 12:739–741 (1972).
34. Sangwan, N.K., Gupta, K., and K.S. Dhindsa, Fatty Acid Composition of Developing Soybeans, *J. Agric. Food Chem.* 34:415–417 (1986).
35. Mohamed, A.I., T. Mebrahtu, F.M. Hashem, and R.B. Dadson, Accumulation Rate of Oil, Unsaturated Fatty Acids and Lipoyxygenase in Vegetable Soybean, in *Proceedings of the Annual Meeting of ASA, CSSA, and SSA*, Salt Lake City, UT, Oct. 31–Nov. 4, 1999, p. 121.
36. Yazdi-Samadi, B., R.W. Rinne, and R.D. Steif, Components of Developing Soybean Seeds: Oil, Protein, Sugars, Starch, Organic Acids and Amino Acids, *Agron. J.* 69:481–486 (1977).
37. Minamide, T., and A. Hata, Effect of Harvest Time and Storage Temperature on the Quality of Green Soybean Seeds (Edamame), *Kyoto-furisu Daigaku Gakujutsu Hokoku, Rigaku, Seikatsu Kagaku (Jpn.)* 41:23–28 (1990).
38. Liu, K., and P. Markakis, Effect of Maturity and Processing on the Trypsin Inhibitor and Oligosaccharides of Soybeans, *J. Food Sci.* 52(1):222–223, 225 (1987).
39. Tsou, S.C.S., and T.L. Hong, Application of NTRS for Quality Evaluation of Soybean and Vegetable Soybean, in *Proceedings of the Symposium on Improving Nutrition Through Soybean*, Jiin, China, 1990.
40. Miyazaki, S., K. Yagasaki, and T. Yasui, The Rapid Determination of Starch in Soybean Seeds with Iodine-Starch Staining, *Jap. J. Crop Sci.* 54:177–178, (1985).
41. Nakayama, M., *Technical Report of New Sweetener*, edited by T. Masai, Dauchi International Co. Ltd., 1987, pp. 151–166.
42. Mohamed, A., Nutritional and Health Benefits of Vegetable Soybean: Beyond Protein and Oil, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Tacoma, Washington, Aug. 10–12, 2001, pp. 131–134.
43. Lee, I.B., and K.W. Chang, Changes in Concentration of Tocopherols and Fatty Acids During Germination and Maturation of Soybean (*Glycine max*), *Han'guk Nonghwa Hakhoechi* 36(2):127–133 (1993).
44. Almonor, G.O., G.P. Fenner, and R.F. Wilson, Temperature Effects on Tocopherol Composition in Soybeans with Genetically Improved Oil Quality, *J. Am. Oil Chem. Soc.* 75:591–596 (1998).
45. Bates, R.P., and R.F. Matthews, *Proc. Fla. State Hort. Soc.* 88:266–271 (1975).
46. Collins, J.L., and G.G. Sanders, Changes in Trypsin Inhibitory Activity in Some Soybean Varieties During Maturation and Germination, *J. Food Sci.* 41:168–172 (1976).
47. Liu, K., Effects of Processing and Maturity on Certain Antinutritional Factors in Soybeans, M.S. Thesis, Michigan State University, East Lansing, Michigan, 1986.
48. Yao, J.J., L.S. Wei, and M.P. Steinberg, Effect of Maturity on Chemical Composition and Storage Stability of Soybeans, *J. Am. Oil Chem. Soc.* 60(7):1245–1249 (1983).
49. Tanimura, W., I. Kamoi, and T. Obara, Distribution of Trypsin Inhibitors in Green Soybean (Edamame) During Cultivation, *Nippon Shokuhin Kogyo Gakkaishi* 27:245–251 (1980).

50. Reddy, N.R., S.K. Sathe, and D.K. Salunkhe, Phytates in Legumes and Cereals, *Adv. Food Res.* 28:1–92 (1982).
51. Mebrahtu, T., A. Mohamed, and A. Elmi, Accumulation of Phytate in Vegetable-Type Soybean Genotypes Harvested at Four Developmental Stages, *Plant Foods Hum. Nutr.* 50:179–187 (1997).
52. Kudou, S., Y. Fleury, D. Welti, D. Magnolato, T. Uchida, K. Kitamura, *et al.*, Malonyl Isoflavone Glycosides in Soybean Seeds (*Glycine max* Merrill), *Agric. Biol. Chem.* 55:2227–2233 (1991).
53. Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, *et al.*, Factors Affecting Isoflavone Content in Soybean Seeds: Changes in Isoflavones, Saponins, and Composition of Fatty Acids at Different Temperatures During Seed Development, *J. Agric. Food Chem.* 43:1184–1192 (1995).
54. Joseph, A.H.M., W.R. Fehr, P.A. Murphy, and G.A. Welke, Influence of Genotype and Environment on Isoflavone Contents of Soybean, *Crop Sci.* 40:48–51 (2000).
55. Hwang, J., H.N. Hodis, and A. Sevanian, Soy and Alfalfa Phytoestrogen Extracts Become Potent Low-Density Lipoprotein Antioxidants in the Presence of Acerola Cherry Extract, *J. Agric. Food Chem.* 49:308–314 (2001).
56. Hsu, C.S., W.W. Shen, Y.M. Hsueh, and S.L. Yeh, Soy Isoflavone Supplementation in Postmenopausal Women: Effects on Plasma Lipids, Antioxidant Enzyme Activities and Bone Density, *J. Reprod. Med.* 46:221–226 (2001).
57. Shiraiwa, M., K. Harada, and K. Okubo, Composition and Content of Saponins in Soybean Seed According to Variety, Cultivation Year and Maturity, *Agric. Biol. Chem.* 55:323–331 (1991).
58. Tsukamoto, C., A. Kikuchi, K. Harada, K. Kitamura, and K. Okubo, Genetic and Chemical Polymorphisms of Saponins in Soybean Seed, *Phytochemistry* 34:1351–1356 (1993).
59. Okubo, K., M. Iijima, Y. Kobayashi, M. Yoshikoshi, T. Uchida, and S. Kudou, Components Responsible for the Undesirable Taste of Soybean Seeds, *Biosci. Biotechnol. Biochem.* 56:99–103 (1992).
60. Tsukamoto, C., A. Kikuchi, S. Kudou, K. Harada, T. Iwasaki, and K. Okubo, Genetic Improvement of Saponin Components in Soybean, *ACS Symp. Ser.* 546:373–379 (1994).
61. Iijima, M., K. Okubo, F. Yamauchi, H. Hirono, and M. Yoshikoshi, Effect of Glycosides Like Saponin on Vegetable Food Processing, Part II: Undesirable Taste of Glycosides Like Saponins, in *Proceedings Papers*, International Symposium on New Technology of Vegetable Protein, Oils and Starch Processing, Chinese Cereals and Oil Association, Beijing, China, 1987, Vol. 2, pp. 109–123.
62. Kitagawa, I., T. Taniyama, Y. Nagahama, K. Okubo, F. Yamauchi, and M. Yoshikawa, Saponin and Sapogenol, XLII: Structures of Acetyl-Soyasaponins AI, A2, and 41, Astringent Partially Acetylated Bisdesmosides of Soyasapogenol A, from American Soybean, the Seeds of *Glycine max* Merrill, *Chem. Pharm. Bull. (Tokyo)* 36:2819–2828 (1988).
63. Taniyama, T., Y. Nagahama, M. Yoshikawa, and I. Kitagawa, Saponin and Sapogenol, XLIII: Acetyl-Soyasaponins &, &, and &, New Astringent Bisdesmosides of Soyasapogenol A, from Japanese Soybean, the Seeds of *Glycine max* Merrill, *Chem. Pharm. Bull. (Tokyo)* 36:2829–2839 (1988).
64. Kudou, S., M. Tonomura, C. Tsukamoto, T. Uchida, T. Sakabe, N. Tamura, *et al.*, Isolation and Structural Elucidation of DDMP-Conjugated Soyasaponins as Genuine Saponins from Soybean Seeds, *Biosci. Biotechnol. Biochem.* 57:546–550 (1993).

65. Fenwick, G.R., K.R. Price, C. Tsukamoto, and K. Okubo, Saponins, in *Toxic Substances in Crop Plants*, edited by J.P.F. D'Mello, C.M. Duffus, and J.H. Duffus, The Royal Society of Chemistry; Cambridge, U.K., 1991, Chapter 12.
66. Arditì, T. T. Meredith, and P. Flowerman, Renewed Interest in Soy Isoflavones and Saponins, *Cereal Foods World* 45:414–417 (2000).
67. Nakashima, H., K. Okubo, Y. Honda, T. Tamura, S. Matsuda, and Y.N. Amamoto, Inhibitory Effect of Glycosides Like Saponin from Soybean on the Infectivity of HIV *in vitro*, *AIDS (Lond.)* 3:655–658 (1989).
68. Konoshima, T., and M. Kozuka, Constitutions of Leguminous Plants, XIII: New Triterpenoid Saponins from *Wisteria brachybotrys*, *J. Nutr. Prod.* 54:830–836 (1991).
69. Tsukamoto, C., A. Kikuchi, S. Kudou, K. Harada, K. Kitamura, and K. Okubo, Group A Acetyl Saponin-Deficient Mutant from the Wild Soybean, *Phytochemistry* 31:4139–4142 (1992).
70. Tsukamoto, C., A. Kikuchi, Y. Shimamoto, J. H. Kim, K. Harada, N. Kaizuma, *et al.*, The Frequency and Distribution of Polymorphisms of Soybean Seed Saponins, and Identification of a Soyasapogenol A Deficient Mutant, *Jpn. J. Breed.* 43(Suppl. 2):161 (1993b).
71. Mohamed, A.I., and M. Rangappa, Nutrient Composition and Antinutritional Factors in a Vegetable Soybean (*Glycine max* (L.) merr): Genotypes, II: Oil, Fatty Acids, Sterols, and Lipooxygenase Activity, *Food Chem.* 44:277–282 (1992).
72. Ibrahim, N., R.K. Puri, S. Kapila, and N. Unklesby, Plant Sterols in Soybean Hull, *J. Food Sci.* 55:271–272 (1990).
73. Everson, G.J., H. Steenbock, D.C. Cederquist, and H.T. Parsons, The Effect of Germination, the Stage of Maturity, and the Variety upon the Nutritive Value of Soybean Products, *J. Nutr.* 27:225–229 (1944).
74. Standal, R.B., Nutritional Value of Proteins of Oriental Soybean Foods, *J. Nutr.* 81:279–285 (1963).
75. Mbuvi, S.W., and J.B. Litchfield, Green Soybeans as Vegetable: Comparing Green Soybeans with Green Peas and Lima Beans, and Maximized Harvest Time Determinations Using Mathematical Modeling, *J. Veg. Crop Prod.* 1:99–121 (1995).
76. Horlings, G.P., E.E. Gamble, and S. Shanmugasundaram, Weathering of Soybean [*Glycine max* L.] in the Tropics, as Affected by Seed Characteristics and Reproductive Development, *Trop. Agric.* 71:110–115 (1994).
77. Iwata, T., H. Sugiura, and K. Shirahata, Keeping Quality of Green Soybeans by Whole Plant Packaging, *J. Jap. Soc. Hort. Sci.* 51:224–230 (1982).
78. Rackis, J.J., D.H. Honig, D.J. Sessa, and H.A. Moser, Lipooxygenase and Peroxidase Activities of Soybeans as Related to the Flavor Profile During Maturation, *Cereal Chem.* 49:587–597 (1972).
79. Okubo, K., Dry Mouth Feel, Undesirable Components of Soybean and Behavior of the Components on Soybean Food Processing, *Nippon Shokuhin Kogyo Gakkaishi* 35:866–874 (1988).
80. Sugawara, E., T. Ito, T. Odagiri, K. Kubota, and A. Kobayashi, Changes in Aroma Components of Green Soybeans with Maturity, *Nippon Noeikagaku Kaishi* 62:149–155 (1988).
81. Maga, J.A., Review of Flavor Investigations Associated with the Soy Products, Raw Soybeans, Defatted Flakes and Flours, and Isolates, *J. Agric. Food Chem.* 21:864–868 (1973).
82. Mebrahtu, T., and T. Andebrhan, Diallel Analysis of Vegetable Soybean, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, 2001.

83. Kitamura, K., Genetic Improvement of Nutritional and Food Processing Quality in Soybean, *JARQ* 29:1-8 (1995).
84. Takahashi, N., Vegetable Soybean Varietal Improvement in Japan—Past, Present, and Future, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taipei, Taiwan, 1991, pp. 26-29, p. 151.
85. Mebrahtu, T., A. Mohamed, C.Y. Wang, and T. Andebrhan, Analysis of Isoflavone Contents in Vegetable Soybeans, *Plant Foods Hum. Nutr.* 59:1-7, (2004).
86. Wang, C.Y., M.S. Sherrard, S. Pagadala, R.Wixon, and R.A. Scott, Isoflavone Content Among Maturity Group 0 to II Soybeans, *J. Am. Oil Chem. Soc.* 77:483-487 (2000).
87. Wang, H.J., and P.A. Murphy, Isoflavone Content in Commercial Soybean Foods, *J. Agric. Food Chem.* 42:1674-1677 (1994).
88. Anonymous. Available at www.msucare.com/crops/soybeans/maturity.html. Accessed July 9, 2004.
89. Miles, C.A., and M. Sonde, Edamame Variety Trial, Washington State University, Vancouver Research and Extension Unit, 2004: www.Agsyst.wsu.edu/
90. Chen, K., S.H. Lai, and S. Cheng, Vegetable Soybean Seed Production Technology in Taiwan, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taipei, Taiwan, 1991, pp. 45-52.
91. Sitatani, K., Cultivation Practices for Vegetable Soybean, in *Vegetable Soybean Production: Proceedings of a Training Course*, edited by M. Shanmugasundaram, Chiang Mai, Thailand, February 18-24, 1991, Publication No. 92-369, Asian Vegetable Research and Development Center, Taipei, Taiwan, 1992, pp. 19-23.
92. Mohamed, A.I., T. Mebrahtu, and M.S.S. Rao, Green Vegetable Soybean as Functional Food, *Inform* 11(6):S83 (2000).
93. Eldridge, A., and W. Kwolek, Soybean Isoflavones: Effect of the Environment and Variety on Composition, *J. Agric. Food Chem.* 31:394-396 (1983).
94. Mebrahtu, T., T. Andebrhan, and A.I. Mohamed, Agronomic and Nutritional Evaluation of Vegetable Soybean, *Va. J. Sci.* 51(2):70 (2000).
95. Horlings, G.P., E.E. Gamble, and S. Shanmugasundaram, The Influence of Seed Size and Seed Coat Characteristics on Seed Quality of Soybean in the Tropics, II: Simulating Weathering, *Seed Sci. Technol.* 19:665-685 (1991).
96. Akazawa, T., and T. Fukushima, Relationship of Varietal Traits and Cultivating Conditions to the Content of Several Ingredients in Green Soybeans (Edamame), *Yamagata Daigaku Kiyo Mogaku (Jpn.)* 11:415-421 (1991).
97. Britz, S.J., Environmental Signals Triggering Enhanced Content of Vitamin E in Seeds of Vegetable Soybean Varieties: Implications for Global Change, The Second World Soybean Conference, 2001.
98. Kamiyama, Y., Vegetable soybean seed production technology in Japan, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taipei, Taiwan, 1991, pp. 43-44.
99. Miles, C.A., and L. Zenz, Edamame Production for SW Washington, 2004: www.gsyst.wsu.edu/
100. Ciba, Y., Postharvest Processing, Marketing, and Quality Degradation in Vegetable Soybean in Japan, in *Vegetable Soybean: Research Needs for Production and Quality*

- Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taipei, Taiwan, 1991, pp. 108–112.
101. Chotiyanwong, P., and A. Chotiyanwong, Postharvest Management of Vegetable Soybean, in *Vegetable Soybean Production: Proceedings of a Training Course*, edited by M. Shanmugasundaram, Chiang Mai, Thailand, February 18–24, 1991, Publication No. 92-369, Asian Vegetable Research and Development Center, Taipei, Taiwan, 1992, pp. 24–26.
 102. Tanusi, S., Changes of Carbohydrate Contents of the Soybean Seed (Cotyledon, Hull, and Hypocotyl) During Growth, *J. Jpn. Soc. Food Nutr.* 25:89–93 (1972).
 103. Shanmugasundaram, S., and M.-R. Yan, Mechanization of Vegetable Soybean Production in Taiwan, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, WA, 2001, pp. 167–172.
 104. Hsieh, C.-C., and C.-S. Su, Management Inputs and Mechanical Harvesting of Vegetable Soybean in Taiwan, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taipei, Taiwan, 1991, pp. 61–64.
 105. Osodo, K., *Technology of Quality Maintenance and Storage for Vegetables*, Vol. 54, Practical Report, Ministry of Agriculture, Forestry and Fisheries, 1978, pp. 1–39.
 106. AVRDC, *Vegetable Soybean Production: Proceedings of a Training Course*, Chiang Mai, Thailand, February 18–24, 1991, Publication No. 92-369, Asian Vegetable Research and Development Center, Taipei, Taiwan, 1992, p. 62.
 107. Akimoto, K., and S. Kuroda, Quality of Green Soybeans Packaged in Perforated PE/PP Film, *J. Jpn. Soc. Hort. Sci.* 50:100–107 (1981).
 108. Iwata, T., and K. Shirahata, Keeping Quality of Green Soybeans, *J. Jpn. Soc. Hort. Sci.* 48:106–113 (1979).
 109. Tsay, L., and S. Sheu, Effects of Cold Storage and Precooling on the Quality of Soybean, in *Vegetable Soybean: Research Needs for Production and Quality Improvement*, edited by S. Shanmugasundaram, Asian Vegetable Research and Development Center (AVRDC), Taipei, Taiwan, 1991, pp. 113–119.
 110. Tsay, L.M., S.C. Sheu, and M.C. Wu, Studies on the Quality Changes of Green Soybean During Storage, *J. Chin. Soc. Hort. Sci.* 36:210–222 (1990).
 111. Hsieh, J. F., and K.H. Tsay, Study of Post-Shelling Treatment for Vegetable Soybean, *1985 Report on Agricultural Machinery Research, Development and Demonstration*, 1985, pp. 116–118.
 112. Murphy, P.A., Isoflavones and Saponin Contents of Edamame, in *Second International Vegetable Soybean Conference*, compiled by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, 2001 (Proceeding CD).
 113. Wang C.Y., Q.M.A. Pagadala, S.M.S. Sherrard, and P. Krishnan, Changes of Isoflavone During Processing of Soy Protein Isolates, *J. Am. Oil Chem. Soc.* 75:337–341 (1998).
 114. Anderson, R.L., and W.J. Wolf, Compositional Changes in Trypsin Inhibitors, Phytic Acid, Saponins and Isoflavones Related to Soybean Processing, *J. Nutr.* 125:581S–588S (1995).
 115. Konovsky, J., D.W. Evans, and T.A. Lumpkin, Heritability of Yield, Plant Architecture, and Quality Traits of Edamame: The Vegetable Soybean, *Soybean Genet. Newslett.* 23:243–249 (1996).

116. Sabota, C., and G. Sharma, Production Potential of Exotic Vegetables in the Southeastern United States, *J. Sust. Agric.* 7:25–39 (1995).
117. Mohamed, A.I., T. Mebrahtu, J.M. Hibbert, and C.Y. Wang, Variability in Vitamin E and Phytosterols Contents of Immature Vegetable-Type Soybeans, in *Proceedings of World Soybean Research Conference VI*, 1999, p. 719.
118. Shiraiwa, M., F. Yamauchi, K. Harada, and K. Okubo, Inheritance of “Group A Saponin” in Soybean Seed, *Agric. Biol. Chem.* 54:1347–1352 (1990).

Chapter 12

Tempeh as a Functional Food

M.J.R. Nout and J.L. Kiers

Wageningen University, Wageningen, The Netherlands, and Friesland Coberco Dairy Foods, Leeuwarden, The Netherlands

Tempeh is a fungal fermented soybean food originating from Indonesia but increasingly known internationally. It is produced by a process involving dehulling, soaking, cooking, and fermenting soybeans by fungal solid-state fermentation. The fungal enzyme activity causes significant decomposition of polymeric components, as well as a considerable modification of soybean flavonoids. As a result, tempeh offers a number of proven health benefits including excellent digestibility and protection against diarrhea and chronic degenerative diseases. Tempeh also gains importance as an interesting food-grade ingredient for formulated functional foods.

Production of Tempeh

Tempeh (also spelled “tempe”) is a collective name for a sliceable mass of precooked fungal fermented beans, cereals, or some other by-products of food processing bound together by the mycelium of a living mold (mostly *Rhizopus* spp.). Yellow-seeded soybeans are the most common and preferred raw material used to make tempeh (1–4). Figure 12.1 shows a cross section of soybean tempeh, as sold in the Netherlands.

The process of tempeh manufacture is shown in Figure 12.2. Tempeh making involves dehulling of soybeans (the most common starting material), soaking in



Figure 12.1. Cross section of tempeh showing the fungal mycelium penetrating the mass of soybeans.

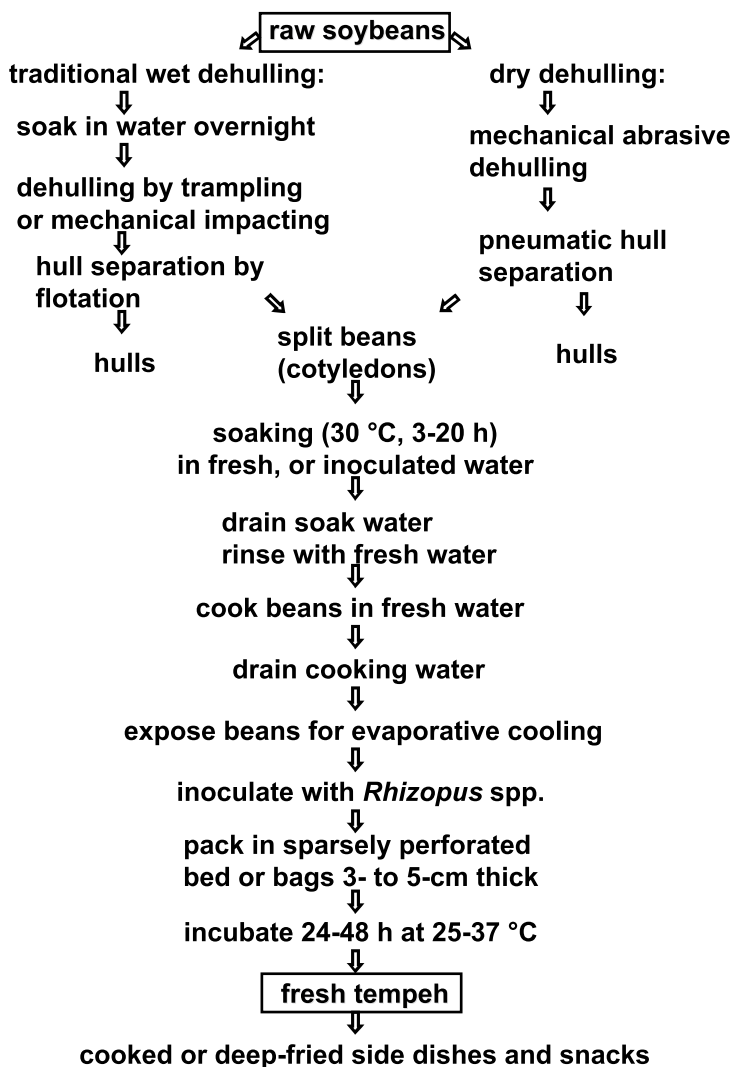


Figure 12.2. Simplified process diagram of tempeh manufacture.

water, boiling in fresh water, inoculation with fermentation starter, and solid-state fermentation of beds of inoculated beans. After incubation periods of typically 2 days at 30°C, fresh tempeh can be harvested and processed into meal components, snacks, or dehydrated to obtain powdered protein enrichment.

A wide variety of microorganisms is involved in the fermentation step of tempeh production. During the soaking stage, bacterial activity is fueled by the water-soluble matter leaching from the beans. During the solid-state fermenta-

tion, molds (especially *Rhizopus oligosporus*, *R. oryzae*, and *Mucor indicus*) are responsible for texture and flavor, but most importantly for the enzyme activities that are expressed. Important enzymes include carbohydrases (5) degrading fiber, proteases (6), and lipases (7). As a result of these enzymatic activities, the cooked beans undergo significant biochemical modifications, which improve the taste and flavor, as well as the functional properties of the product (Table 12.1). With its high protein content (40–50% of dry matter) it serves as a tasty protein complement to starchy staple foods such as rice, and it can replace meat or fish in the diet. In Indonesia, the estimated consumption ranges from 19–34 grams per day per person (8). Tempeh is not consumed raw, but is heated first to develop meat-like flavors, for example, by frying spiced and salted slices in oil, by boiling with coconut milk in soups, by stewing, by roasting spiced kebobs, and by grinding into peppered ground pastes.

Functional Properties

History of Use

Tempeh has evolved as a traditional meat alternative in Indonesia. It was locally known for its easy digestibility, and there is anecdotal evidence that during World War II, prisoners of war suffering from dysentery could not tolerate soybeans but were able to subsist on tempeh; this underscores the easy digestibility of tempeh. During the 1960s, tempeh turned global and became a favorite of vegetarians. Nowadays, increasing numbers of nonvegetarian consumers include it in the diet for the purpose of variation and to reduce the number of “meat-days.” Local expe-

TABLE 12.1
Nutrient Comparison of Tempeh and Chicken Egg and Vitamin Synthesis in Tempeh during Its Fermentation

Composition (% product)	Tempeh	Chicken Egg
dry matter	34–40	25
(% dry matter basis)		
Crude protein	53	52
Crude lipid	20	44
Crude fiber	8.6	—
cholesterol	—	0.6
Energy, MJ/kg	18.9	25.6
	Cooked soybeans	Tempeh
Riboflavin (vitamin B ₂)	1.5 ppm	6.5 ppm (× 4.4)
Nicotinic acid	6.7	25.2 (× 3.8)
Pyridoxine (B ₆)	1.8	8.3 (× 4.6)
Folic acid	0.25	1.0 (× 4.0)

rience in Indonesia shows that addition of tempeh to the diet of (young) diarrhea patients shortens the recovery period (9) after the disease.

Predigestion of Nutrients

The easy digestibility of tempeh is related to the enzymatic degradation of soybean polymeric substances resulting in soluble solids, such as soluble nitrogenous compounds. Macromolecules are degraded into oligomeric and smaller units, which improves tempeh digestion (10). Digestibility of cereals and legumes increases during cooking, and continues to increase during fermentation (11). Cooking improved the total *in vitro* digestibility of both soybean (from 37% to 45%) and cowpea (from 15% to 41%). Subsequent fungal fermentation increased total digestibility only about 3% for both soybean and cowpea. Digestibility was influenced by fungal strain and fermentation time. Although total digestibility of cooked legumes was only slightly improved by mold fermentation, the level of nonfat water-soluble dry matter of food samples increased spectacularly from 4% up to 17% for soybean and from 4% up to 24% for cowpea (Table 12.2). This illustrates that mold fermentation already “predigests” the soybean macronutrients to a significant extent. Fermentation was nearly capable of increasing nutrient availability to the level obtained after *in vitro* digestion of cooked soybeans. *In vivo* trials with rats and piglets show evidence of increased protein digestibility, increased protein efficiency ratio and net protein utilization (12), and higher uptake of total solutes (13).

Antimicrobial Effects

Tempeh was reported to contain an antibacterial substance, confirmed by demonstrated antimicrobial activity against selected species of Gram-positive bacteria (14–16). Recent work shows that several tempeh extracts were able to inhibit adhesion of *E. coli* to piglet small intestinal brush border membranes *in vitro* (Fig. 12.3) and might therefore have a protective effect against *E. coli* infection (16).

TABLE 12.2
Changes in *In Vitro* Absorbability and Digestibility as a Result of Tempeh Fermentation (11)

	Absorbability (% of fat-free dm)	Digestibility (% of fat-free dm)	A/D (%)
Cooked soybean	4.8	22.3	22
Mold strain 575, 24h fermented	6.1	23.7	64
Mold strain 575, 44h fermented	16.7	26.1	64
Mold strain 582, 24h fermented	16.4	26.2	63
Mold strain 582, 44h fermented	14.0	27.2	51

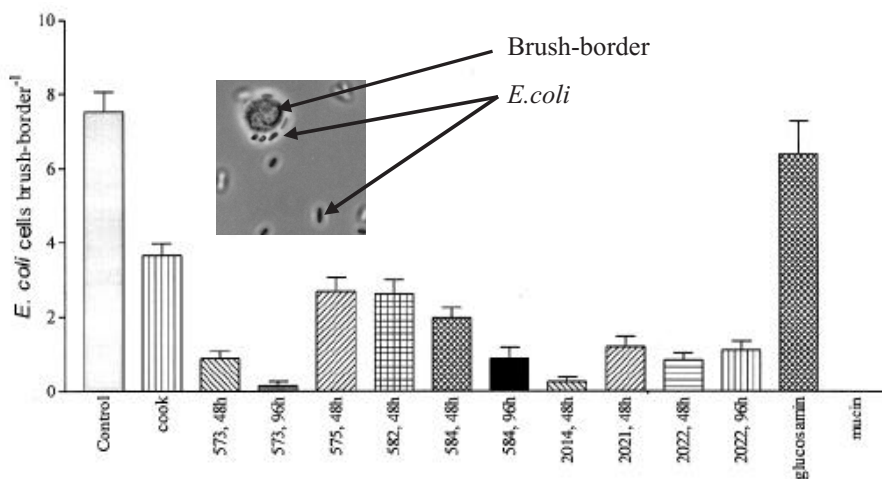


Figure 12.3. *In vitro* inhibition of adhesion of enterotoxigenic *Escherichia coli* to intestinal brush border membranes (16).

Protection against Diarrhea

In rabbits and piglets, diarrhea caused by *E. coli* was reduced by tempeh. These findings correlate with a protective effect against fluid losses found in small intestinal segment perfusion experiments (13) in piglets. Tempeh appeared to contain a high-molecular-weight fraction (> 5 kDa) that protected against fluid losses induced by ETEC. Tempeh can be very useful as a nutritional supplement in oral rehydration therapy, and in cases of (post-weaning) diarrhea, for accelerating the recovery of young animals and young children, who are most at risk for enterotoxic diarrhea and malnutrition. The effect on the occurrence and severity of diarrhea in ETEC K88+–challenged weaned piglets was determined by Kiers *et al.* (17). Severity of diarrhea was significantly less on the diet containing tempeh compared with the control diet containing toasted soybeans. Various beneficial effects of tempeh in disease prevention and treatment, principally in diarrhea management, and positive nutritional impact in Indonesian children have been reported (18–20). An immune modulating effect was suggested, but further evidence for this phenomenon will have to be sought (21).

Intestinal Growth and Proliferation

Weaning is often associated with marked histological and biochemical changes of the small intestine, causing decreased digestive and absorptive capacity and contributing to post-weaning diarrhea. Biopsies from the human small intestinal mucosa showed improved repair after intestinal inflammation as a result of tempeh supplementation (9). In a trial with piglets, no indication of beneficial effects of tempeh on

maintaining or quickly restoring villous height in piglets after weaning was observed (J.L. Kiers *et al.*, unpublished data).

Antioxidative Properties of Fermented Soybeans

Soybeans contain natural antioxidants. It is interesting to note that fermented soyfoods do not lose their antioxidative properties, but in contrast show increased antioxidative capacity (22). The four important aglycones in tempeh are genistein, daidzein, glycitein, and factor 2 (6,7,4'-trihydroxyisoflavone) (23). Another antioxidative substance in tempeh was identified as 3-hydroxyanthranilic acid (HAA); this was not detected in unfermented beans (24) and was formed only as a result of fungal fermentation. Of several soybean foods, tempeh had somewhat lower isoflavone content than tofu but contained elevated levels of the aglycones formed by enzymatic hydrolysis during fermentation (25,26). Fermentation of soy increased the human bioavailability of isoflavones. This was shown *in vivo*: eight women aged 20–41 years retained approximately 75% of isoflavones (daidzein and genistein) from soyfoods including tempeh (27).

Chronic Degenerative Diseases

Besides the role of antioxidants in protecting foods against oxidative spoilage, antioxidants in soybeans (and tempeh) are of interest with respect to their protective role against oxidative stress known to be involved in the pathogenesis of various chronic degenerative diseases such as cancer, coronary diseases, osteoporosis, and menopausal symptoms. Soybean protein has been known for many years to have a hypocholesterolemic effect. It is therefore not surprising that tempeh has also been found to lower blood cholesterol levels (28) and may therefore be of benefit as a protective agent against cardiovascular disease. In a number of clinical intervention trials, total cholesterol and low-density lipoprotein (LDL) cholesterol were significantly reduced in subjects treated with tempeh, whereas high-density lipoprotein (HDL) cholesterol was raised (19,29,30). It was demonstrated that tempeh, especially its glucolipids, inhibits the proliferation of tumour cells in mice (31,32). In Southeast Asia, Indonesians are undoubtedly the largest consumers of tempeh, as well as of tofu (locally called *tahu*). Epidemiological studies relating to tempeh consumption and the prevalence of cancer, particularly in Indonesia, have not yet been conducted.

Novel Applications

In addition to its traditional use in both Oriental and Western cuisine, tempeh can be processed into powdered form for convenient use in formulated foods and feeds. The use of tempeh in the rehabilitation of children suffering from protein-energy malnutrition in Indonesia was shown to have a greater nutritional impact than food mixtures containing cooked but unfermented soybeans. Protein-energy malnutrition is highly prevalent in developing countries due to the decline in breast-feeding, use of complementary foods that are low in energy and nutrients, and a high prevalence of diarrhea and infections (33). Fermentation of soybean-cereal mixtures has great potential for application in comple-

mentary foods. Because of their nutritional relevance, mixtures of cereals and leguminous seeds, such as finger millet with various legumes (34), maize and soybean, rice and black beans (35), and sorghum and common bean have been evaluated. The nutritional potential and superior digestibility make tempeh a valuable enrichment for starch-based formulated foods, such as infant porridges (36), among others. A significantly higher growth rate, shorter duration of diarrheal episodes, and shorter rehabilitation period was reported in children suffering from protein-energy malnutrition who were given a porridge containing tempeh and yellow maize, compared to those fed a similar porridge made of milk and yellow maize (37). Functional properties of tempeh will be of interest in the areas of diarrhea management, nutritional recovery of compromised patients, and health foods (38), as well as in specialized feeds such as weaning formula for piglets.

References

1. Ko, S.D., and C.W. Hesseltine, Tempe and Related Foods, in *Microbial Biomass*, edited by A.H. Rose, Academic Press, London, 1979, Vol. 4, pp. 115–140.
2. Nout, M.J.R., and F.M. Rombouts, Recent Developments in Tempe Research, *J. Appl. Bacteriol.* 69:609–633 (1990).
3. Steinkraus, K.H., *Handbook of Indigenous Fermented Foods* (2nd ed.), Marcel Dekker, New York, 1995.
4. Nout, M.J.R., and J.L. Kiers, Tempe Fermentation, Innovation and Functionality: Up-date into the 3rd Millenium, *J. Appl. Microbiol.*, in press.
5. Sarrette, M., M.J.R. Nout, P. Gervais, and F.M. Rombouts, Effect of Water Activity on Production and Activity of *Rhizopus oligosporus* Polysaccharidases, *Appl. Microbiol. Biotechnol.* 37:420–425 (1992).
6. Baumann, U., and B. Bisping, Proteolysis during Tempe Fermentation, *Food Microbiol.* 12:39–47 (1995).
7. Ruiz-Teran, F., and J.D. Owens, Chemical and Enzymic Changes during the Fermentation of Bacteria-Free Soya Bean Tempe, *J. Sci. Food Agric.* 71:523–530 (1996).
8. Sayogyo, S., Tempe in the Indonesian Diet (abstract), in *Second Asian Symposium on Non-salted Soybean Fermentation*, edited by H. Hermana, M.K.M.S. Mahmud, and D. Karyadi, Nutrition Research and Development Centre, Jakarta, Indonesia, 1990, p. 17
9. Sudigbia, I., Tempe in the Management of Infant Diarrhea in Indonesia, in *The Complete Handbook of Tempe*, edited by J. Agranoff, American Soybean Association, Singapore, 1999, pp. 33–40.
10. Matsuo, M., Digestibility of Okara-Tempe Protein in Rats, *J. Jpn. Soc. Food Sci. Technol. [Nippon Shokuhin Kagaku Kogaku Kaishi]* 43:1059–1062 (1996).
11. Kiers, J.L., M.J.R. Nout, and F.M. Rombouts, *In Vitro* Digestibility of Processed and Fermented Soya Bean, Cowpea and Maize, *J. Sci. Food Agric.* 80:1325–1331 (2000).
12. Tchango, J.T., The Nutritive Quality of Maize-Soybean (70:30) Tempe Flour, *Plant Foods Hum. Nutr.* 47:319–326 (1995).
13. Kiers, J.L., M.J.R. Nout, F.M. Rombouts, M.J.A. Nabuurs, and J. Van der Meulen, Protective Effect of Processed Soya Bean during Perfusion of ETEC-Infected Small Intestinal Segments of Early-Weaned Piglets, in *8th Symposium on Digestive Physiology in Pigs*, Uppsala, Sweden, 2000.
14. Rachmaniar, R., and E. Siregar, A Preliminary Study on the Chemical Composition of Tempe Extract as an Antimicrobial Activity (abstract), in *Second Asian Symposium on*

- Non-salted Soybean Fermentation*, edited by H. Hermana, M.K.M.S. Mahmud, and D. Karyadi, Nutrition Research and Development Centre, Jakarta, Indonesia, 1990, p.10.
15. Kobayasi, S.Y., N. Okazaki, and T. Koseki, T., Purification and Characterization of an Antibiotic Substance Produced from *Rhizopus oligosporus* IFO 8631, *Biosci. Biotechnol. Biochem.* 56:94–98 (1992).
 16. Kiers, J. L., M.J.R. Nout, F.M. Rombouts, M.J.A. Nabuurs, and J. Van der Meulen, Inhibition of Adhesion of Enterotoxigenic *Escherichia coli* K88 by Soya Bean Tempe, *Lett. Appl. Microbiol.* 35:311–315 (2002).
 17. Kiers, J.L., J.C. Meijer, M.J.R. Nout, F.M. Rombouts, M.J.A. Nabuurs, and J. Van der Meulen, Effect of Fermented Soya Beans on Diarrhea and Feed Efficiency in Weaned Piglets, *J. Appl. Microbiol.* 95:545–552 (2003).
 18. Soenarto, Y., I. Sudigbia, H. Hermana, M. Karmini, and D. Karyadi, Antidiarrheal Characteristics of Tempe Produced Traditionally and Industrially in Children Aged 6–24 Months with Acute Diarrhea, in *International Tempe Symposium*, edited by S. Sudarmadji, S. Suparmo, and S. Raharjo, Indonesian Tempe Foundation, Jakarta, Indonesia, Bali, Indonesia, 1997, pp. 174–186.
 19. Karyadi, D., and W. Lukito, Beneficial Effects of Tempeh in Disease Prevention and Treatment, *Nutr. Rev.* 54:S94–S98 (1996).
 20. Karyadi, D., and W. Lukito, Functional Food and Contemporary Nutrition-Health Paradigm: Tempeh and Its Potential Beneficial Effects in Disease Prevention and Treatment, *Nutrition* 16:697 (2000).
 21. Karmini, M., Tempe and Infection, in *The Complete Handbook of Tempe*, edited by J. Agranoff, American Soybean Association, Singapore, 1999, pp. 46–50.
 22. Berghofer, E., B. Grzeskowiak, N. Mundigler, W.B. Sentall, and J. Walcak, Antioxidative Properties of Faba Bean-, Soybean- and Oat Tempeh, *Int. J. Food Sci. Nutr.* 49:45–54 (1998).
 23. Hoppe, M.B., H.C. Jha, and H. Egge, Structure of an Antioxidant from Fermented Soybeans (Tempeh), *J. Am. Oil Chem. Soc.* 74:477–479 (1997).
 24. Esaki, H., H. Onozaki, S. Kawakishi, and T. Osawa, New Antioxidant Isolated from Tempeh, *J. Agric. Food Chem.* 44:696–700 (1996).
 25. Anderson, R.L., and W.J. Wolf, Compositional Changes in Trypsin Inhibitors, Phytic Acid, Saponins and Isoflavones Related to Soybean Processing, *J. Nutr.* 125:S581–S588 (1995).
 26. Wang, H.J., and P.A. Murphy, Mass Balance Study of Isoflavones during Soybean Processing, *J. Agric. Food Chem.* 44:2377–2383 (1996).
 27. Xu, X., H.J. Wang, P.A. Murphy, and S. Hendrich, Neither Background Diet nor Type of Soy Food Affects Short-Term Isoflavone Bioavailability in Women, *J. Nutr.* 130:798–801 (2000).
 28. Guermani, L., C. Villaume, H.M. Bau, J.P. Nicolas, and L. Mejean, Modification of Soyprotein Hypocholesterolemic Effect after Fermentation by *Rhizopus oligosporus* spT3, *Sciences des Aliments* 13:317–324 (1993).
 29. Brata-Arbai, A.M., The Effect of Tempe Diet on Uric Acid and Plasma Lipid Level, in *International Tempe Symposium*, Den Pasar, Bali, Indonesia, Indonesian Tempe Foundation, Jakarta, Indonesia, 1997, pp. 187–198.
 30. Brata-Arbai, A.M., Cholesterol Lowering Effect of Tempe, in *The Complete Handbook of Tempe*, edited by J. Agranoff, American Soybean Association, Singapore, 1999, pp. 51–70.

31. Kiriakidis, S., S. Stathi, H.C. Jha, R. Hartmann, and H. Egge, Fatty Acid Esters of Sitosterol 3 Beta Glucoside from Soybeans and Tempe (Fermented Soybeans) as Antiproliferative Substances, *J. Clin. Biochem. Nutr.* 22:139–147 (1997).
32. Jha, H.C., S. Kiriakidis, M. Hoppe, and H. Egge, Antioxidative Constituents of Tempe, in *International Tempe Symposium*, Den Pasar, Bali, Indonesia, Indonesian Tempe Foundation, Jakarta, Indonesia, 1997, pp. 73–84.
33. Abiodun, P.O., Use of Soya-beans for the Dietary Prevention and Management of Malnutrition in Nigeria, *Acta Paediatr. Scand. Suppl.* 374:175–182 (1991).
34. Mugula, J.K., and M. Lyimo, Evaluation of the Nutritional Quality and Acceptability of Finger millet-Based Tempe as Potential Weaning Foods in Tanzania, *Int. J. Food Sci. Nutr.* 50:275–282 (1999).
35. Rodriguez-Burger, A.P., A. Mason, and S.S. Nielsen, Use of Fermented Black Beans Combined with Rice to Develop a Nutritious Weaning Food, *J. Agric. Food Chem.* 46:4806–4813 (1998).
36. Kodyat, B.A., A. Sukaton, and D. Latief, Traditional Soybean Fermentation (Tempe) for Increasing Nutritional Status of Children in Indonesia, in *Second Asian Symposium on Non-salted Soybean Fermentation*, edited by H. Hermana, M.K.M.S. Mahmud, and D. Karyadi, Nutrition Research and Development Centre, Bogor, Indonesia, 1990, pp. 110–115.
37. Kalavi, F.N.M., N.M. Muroki, A.M. Omwega, and R.K.N. Mwadime, Effect of Tempe Yellow Maize Porridge and Milk Yellow Maize Porridge on Growth Rate, Diarrhoea and Duration of Rehabilitation of Malnourished Children, *East African Med. J.* 73:427–431 (1996).
38. Kiers, J.L., M.J.R. Nout, F.M. Rombouts, B.C. Koops, K.M.J. Van Laere, E. Wissing, R.J.J. Hagemann, and J. Van der Meulen, Process for the Manufacture of a Fermented Health-Promoting Product, European Patent Application No. 01201510.3-2110, Numico Nutrica, October 31, 2001.

Chapter 13

Soy Sauce as Natural Seasoning

KeShun Liu

University of Missouri, Columbia, MO 65211

Soy sauce is a dark brown liquid made from a mixture of soybeans and wheat, mostly through natural fermentation. It is known as *jiangyou* (Mandarin) or *chi-angyu* (Cantonese) in China, meaning oil from *jiang* (a fermented food paste), and *shoyu* in Japan. Discovered in China more than 2,500 years ago, soy sauce is one of the world's oldest condiments. Over the centuries, it has remained a cornerstone of many Asian cuisines by contributing a unique flavor profile to traditional Asian foods. Today, it is becoming increasingly known in the West as natural seasoning that promotes balance among ingredients in food products, and holds great potential as a flavoring and flavor-enhancing material for a wide variety of non-Asian food products (1). Furthermore, soy sauce has strong antioxidant activity as well as some antiplatelet activity and thus can be considered a functional food ingredient (2–4).

This chapter covers one of the major fermented soy foods and the most popular one—soy sauce—with respect to its production, principle of processing, chemical composition, applications in food systems, and health benefits. Additional information can be found in Yokotsuka (5), Liu (6), Anonymous (1), and Huang and Teng (7).

Types of Soy Sauce

There are many types of soy sauce. Based on preparation principles, soy sauce is divided into three groups—fermented soy sauce, chemical soy sauce, and semi-chemical soy sauce. Based on geographical location of original source, there are Chinese and Japanese soy sauces. Based on physical or other properties, there are liquid soy sauce, powdered soy sauce, clear soy sauce, reduced-salt soy sauce, preservative-free soy sauce, and others.

In Japan, based on differences in raw ingredients and conditions of fermentation or duration of aging, fermented soy sauces are further divided into five main types that are officially recognized. *Koikuchi* shoyu is a major type, representing about 85% of total soy sauce production in Japan. Characterized by a strong aroma, myriad flavors, and a deep, red-brown color, it is made from equal amounts of wheat and soybeans in the koji and serves as an all-purpose seasoning. *Usukuchi* shoyu is the second popular type of soy sauce in Japan. Characterized by a lighter, red-brownish color and milder flavor and aroma, it is used commonly as a seasoning for food when the original flavor and color must be preserved. When making this type of soy sauce, the ratio of soybeans to wheat is the same as when making *koikuchi* shoyu, but its

fermentation is controlled so that color development is prevented. In addition, before raw soy sauce is pressed out, a digestion mixture of rice koji is added to the fermented mash to make its flavor bland. The remaining three types of soy sauce are produced and consumed only in isolated localities for special uses in Japan. Among them, *tamari* shoyu is very similar to the traditional Chinese type of soy sauce. It is made by using a koji containing a large proportion of soybeans over wheat. In contrast to *tamari* shoyu, *shiro* shoyu is made from a very high ratio of wheat to soybeans in the koji, and is fermented under conditions that prevent color development. *Saishikomi* shoyu is produced by using equal amounts of wheat and soybeans in the koji. However, raw soy sauce instead of a brine solution is mixed with the koji before the second fermentation.

Production of Fermented Soy Sauce

Just like other types of soy foods, the preparation of soy sauce was once a family art passed down from one generation to the next. At present, production of soy sauce at a domestic level is still popular in some regions of the world, but most is made in commercial plants. There are great variations in methods of making soy sauce, depending on geographic regions and varieties of soy sauce. However, regardless of the level of production and the methods used, the basic steps are the same, including treatment of raw materials, koji making, brine fermentation, pressing, and refining (1,5,8,9). A typical process for *koikuchi* shoyu, the representative Japanese type of soy sauce, is outlined in [Figure 13.1](#).

Treatment of Raw Materials

The initial step is to treat soybeans and wheat simultaneously. Whole soybeans are soaked in water overnight at an ambient temperature, preferably 30°C. To avoid possible growth of undesirable spore-forming *Bacillus*, water must be changed every 2–3 hours. The soaked soybeans are cooked for several hours under steam pressure. At home, soybeans are boiled in an open pan until soft.

Defatted soy products, which are popular, are first moistened by spraying with an amount of water equal to 30% of their weight. This is followed by steam pressure for 45 minutes. The heated soybeans or soy grits are allowed to cool quickly to less than 40°C (9).

Quick cooling of soybeans or soy grits to less than 40°C is accomplished by constant mixing or spreading of the materials in layers of approximately 30 cm on a perforated surface and forcing air through them. Rapid cooling prevents proliferation of unwanted bacteria before controlled fermentation is initiated. It also helps to maintain good nitrogen availability.

Concurrent with the treatment of soybeans, whole kernel wheat is roasted and cracked in rollers into four or five pieces. Roasting leads to Maillard browning reactions that impart a desirable appearance to the end product. Cracking is necessary for the wheat to absorb adequate moisture from the surface of steamed soy

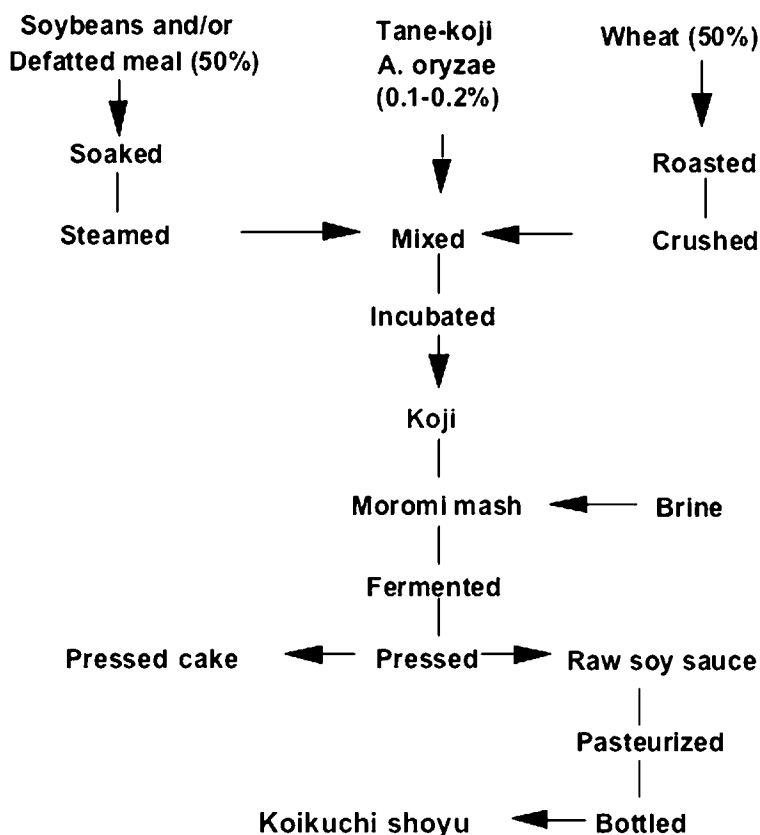


Figure 13.1. Outline of typical preparation process for koikuchi shoyu, the most common Japanese type of soy sauce.

materials. When wheat flour and wheat bran are used, they are steamed after being moisturized.

Kinoshita and colleagues (10) conducted a study to differentiate soy sauce produced from whole soybeans and that from defatted soy meal by analyzing non-volatile components from commercial fermented soy sauces with the use of reversed-phase high-performance liquid chromatography (HPLC). The differences in the two groups were observed in both the factor score plot and the clustering dendrogram of their HPLC profiles. Ferulic acid was identified as one of the key components of the differentiation. This was followed by daidzein and three isoflavone derivatives. All these components showed higher values when soy sauce was produced from whole soybeans.

Chou and Ling (11) examined biochemical changes during aging of soy sauce mash prepared with extruded and traditionally pretreated raw material. They found

that after a 180-day aging period, although not markedly different in pH values, the amounts of total nitrogen, amino nitrogen, free amino acids, and reducing sugars, and the protein utilization rate, were higher in soy sauce prepared with extruded raw material than with traditional raw material. A higher intensity of brown color was also observed in soy sauce prepared with extruded substrate.

Koji Making

Koji is a Japanese word describing a fermented mass made from growing molds on rice, barley, wheat, soybeans, or a combination thereof. The Chinese counterpart for the word *koji* is *qu*, meaning bloom of mold. *Koji* contains a great variety of enzymes that digest starch, protein, and lipid components in raw materials. It is an intermediate product for making not only soy sauce, but also some other fermented products such as fermented soy paste (*jiang* or *miso*), soy nuggets, and Japanese sake.

To make *koji*, we need “*koji* starter.” *Koji* starter, also known as seed *koji*, *koji* seeds, or *tane-koji*, provides spores of microorganisms to make *koji*. The microorganisms found in *koji* starter almost always belong to fungi species, *Aspergillus oryzae* and *A. sojae*. *A. oryzae* molds reproduce only asexually and have the ability to utilize starch, oligosaccharides, simple sugars, organic acids, and alcohols as carbon sources and protein, amino acids, and urea as nitrogen sources. The mold is aerobic, with growth most optimal generally at a pH of 6.0, a temperature of 37°C, and a water content of 50% in a medium. When air supply is limited or water content of the medium is below 30%, its growth slows down. When a temperature is below 28°C, its growth also becomes slow but enzymatic activities remain high.

Since many molds, including *A. oryzae*, are ubiquitous, up until several decades ago wild spores of the species were used as the starter for soy sauce preparation. However, the modern process for making *koji* starter begins with growing a selected *A. oryzae* strain on an agar slant in pure culture. The strain is selected for its special abilities by natural selection or by induced mutation to give a desirable *koji* for a particular fermentation. Therefore, there are many varieties of commercial *tane-koji*, each having a different capacity to break down protein, carbohydrate, and lipid in raw materials. It is very important to select a suitable variety for making a particular product.

To make soy sauce *koji*, the two treated materials (defatted soy flour or whole soybeans and wheat flour) are mixed in a certain proportion, depending on what types of end products are to be made. For example, for *koikuchi* shoyu, the ratio of soybean (or defatted soy meal) to water is about 1:1, whereas for *tamari* shoyu, the ratio is 9:1. The mixture is inoculated with seed *koji* or a pure culture containing *A. oryzae* and *A. sojae*, or one or the other, at a concentration of 0.1–0.2%.

In traditional *koji* making, the inoculated mixture is put into small wooden trays and kept for three or four days in a *koji*-making room. During the mold growth, the temperature and moisture are controlled by manual stirring. In modern *koji* making,

however, the cultured mixture is put into a shallow, perforated vat and kept in a koji room where forced air is circulated and temperature and humidity may thus be controllable (as is the case with an automatic koji-making system). After about three or four days, when the mixture turns green-yellow as a result of sporulation of the inoculated mold, it becomes mature koji.

During koji making, it is advisable to cool the materials twice either by hand mixing or by use of a mechanical device, when their temperature rises to above 35°C or more because of active mold growth. In the early stage of koji making, temperatures as high as 30–35°C are preferable for mycelium growth and the prevention of *Bacillus* as a contaminant. In the latter stage, just before spore formation or after the second cooling, a lower temperature (20–25°C) is necessary to allow maximum production of enzymes. Alternatively, koji may be prepared at a constant low temperature of 23–25°C for a relatively longer time (66 hours).

According to Yokotsuka (5), the major points in koji cultivation include the following: (a) grow as much mold mycelia and as many mold enzymes as possible; (b) maintain a minimal inactivation of enzymes once produced; (c) minimize carbohydrate consumption in raw materials and leave more for subsequent brine fermentation; (d) avoid bacterial contamination in the starting materials and during koji cultivation as much as possible; and (e) shorten the cultivation time with a minimal use of water, electricity, and fuel oil. A soy sauce koji of superior quality should have a dark green color, a pleasant aroma, and a sweet but bitter taste. It also has a high population of yeast, low bacteria counts, and strong activities of proteases and amylases.

Brine Fermentation

Mature koji is now mixed with an equal amount or more (up to 120% by volume) of a salt solution. The mix is allowed to ferment for several months by using osmophilic lactic acid bacteria and yeasts to form a liquid mash known as *moromi* in Japanese. This is the most critical step. During this time, the soybean and wheat transform into a semiliquid, reddish-brown mash. It is this aging process that creates the many distinct flavor and fragrance components that build the soy sauce flavor profile.

There are many factors affecting this critical step of fermentation. The first factor is the salt concentration in the mix. Lower salt concentration promotes growth of undesirable putrefactive bacteria during subsequent fermentation and aging. However, higher salt concentration (in excess of 23%) may retard the growth of desirable halophilic bacteria and osmophilic yeasts. In general, the final concentration of sodium chloride in the mash is in the range 17% to 19%.

Temperature is the next important factor during brine fermentation. In general, the higher the temperature is, the shorter the fermentation time. However, a lower temperature fermentation gives a better product because the rate of enzyme inactivation is slow. A good quality of soy sauce can be made by 6-month fermentation when the temperature of mash is controlled as follows: starting at 15°C for 1 month, followed by 28°C for 4 months, and finishing at 15°C again for 1 month (9).

The ability to control fermentation temperature depends largely on what facility is used. At home, the mix is put in an earthenware crock and the fermentation is under ambient temperatures. In this case, a period of 10–12 months may be necessary for completion of brine fermentation stage. However, on an industrial level, the mash is kept in large wooden containers or concrete vats with aeration devices. The temperature of these surroundings can be controlled mechanically. Thus, fermentation time can be shortened.

During fermentation, occasional brief stirring is required. The purpose of stirring is multiple, as follows: to provide enough aeration for good growth of yeast, to prevent the growth of undesirable anaerobic microorganisms, to maintain uniform temperatures, and to facilitate removal of carbon dioxide generated. However, excessive aeration should be avoided as it will also hinder proper fermentation.

Pressing

After months of fermentation and aging, the mash becomes matured. A perfectly fermented mash should have a bright reddish-brown color, a pleasant aroma, and be salty but tasty. In the case of home processing, raw sauce may be removed from the mash simply by siphoning off from the top or filtering through cloth under a simple mechanical press. In commercial operations, a batch type of hydraulic press is commonly used. Recently, automatic loading of the mash into filter cloth or continuous pressing by a diaphragm-type machine has emerged as an effective method of filtration. The filtrate obtained is stored in a tank to separate the sediments at the bottom and the floating oil on the top.

The insoluble solid contained in the press cake made from soy sauce mash was found to consist of 10% microbial cells, 30% protein, and 20–30% nonproteinaceous substances derived from soybeans and wheat. Among these, the amount of noncellulose polysaccharides is about 7%. It is the presence of such acidic polysaccharide that contributes mainly to the filtration resistance during pressing shoyu mash (12).

Refining

Raw soy sauce may be adjusted to standard salt and nitrogen concentrations. It is then pasteurized at 70–80°C to inactivate enzymes and microorganisms, enhance the unique product aroma, darken the color, and induce the formation of flocs, which facilitate clarification. After heating, the soy sauce is clarified by either sedimentation or filtration. Kaolin, diatomite, or alum may be added to enhance clarification before filtration.

According to Hashimoto and Yokotsuka (13), the heat-coagulating substances produced by heating raw soy sauce are equivalent to 10% of its original volume and 0.025–0.05% of its weight. They consist of 89.1% protein, 9.7% carbohydrate, and 1.2% ash. The major ingredients of the heat-coagulating substances in raw soy sauce are proteins derived from koji enzymes.

The clear supernatant is packed immediately into cans or bottles. In some cases, preservatives such as sodium benzoate and *para*-oxybenzoate may be used. According to Watanabe and Kishi (9), in Japan, the standard amounts for sodium benzoate and *para*-oxybenzoate (mainly butyl ester) are 0.6 g/l and 0.25 g/l respectively.

Principles of Making Fermented Soy Sauce

There are two stages of fermentation occurring in soy sauce preparation. The first fermentation is solid state and occurs during koji making, in which various enzymes are produced under aerobic conditions. The second fermentation occurs after the addition of brine and is known as brine fermentation. It is mainly anaerobic. At the earlier stage of brine fermentation, enzymes from koji hydrolyze proteins to yield peptides and free amino acids. Starch is converted to simple sugars, which in turn serve as substrates for growth of various types of salt-resistant bacteria and yeasts. These organisms become dominant in sequence as fermentation progresses. All these enzymatic and biological reactions, together with concurrent chemical reactions, lead to the formation of many new volatile and nonvolatile substances that contribute to the characteristic color, flavor, and taste of soy sauce (5,14).

Action of Koji Enzymes

During mash fermentation, proteins, carbohydrates, and oil from soybeans and wheat are degraded by protease, peptidase (including glutaminase), and amylase, and lipase, pectinase, and phosphatase derived from koji. According to Komatsu (15), who made soy sauce by fermenting mash initially at 15°C for 30 days, then at 25°C for 120 days, and finally at 28°C for an additional 30 days, as fermentation advances, total nitrogen increased from 0.98 to 1.69 g/100 ml, formyl nitrogen from 0.36 to 0.94 g/100 ml, NH₃ nitrogen from 0.06 to 0.2 g/100 ml, the ratio of formyl nitrogen to total nitrogen from 37.1% to 55.7%, and total nitrogen utilization (total nitrogen in shoyu to total nitrogen in raw materials) increased from 44.7% to 83.1%. At the same time, activities of protease and amylase decreased, and pH also decreased.

Fermentation by Lactic Bacteria and Yeasts

In addition to koji enzyme action, both lactic bacteria and yeasts play an important role in brine fermentation of soy sauce. In the newly produced mash, salt-intolerant lactobacilli and wild yeasts derived from koji are destroyed rapidly and *Bacillus subtilis* remains only as spores. Salt-tolerant micrococci also rapidly disappear because of anaerobic conditions of mash. As a result, the predominant active microbes in shoyu mash are salt-tolerant lactobacilli such as *Pediococcus soyae* (or *P. halophylus*) and yeasts such as *Zygosaccharomyces rouxii* and *Candida (Torulopsis) versatilis* or *C. etchellsii* (5).

P. halophilus grows first during the fermentation, converting simple sugars to lactic acid. The pH of mash decreases from an initial value of 6.5–7.0 to about 5.5. At the same time, production of carbon dioxide will enhance the growth of anaerobic bacteria, which may impart undesirable flavor and aroma. This is why occasional brief aeration by stirring is necessary. As lactic fermentation subsides, *Z. rouxii*, *Torulopsis*, and some other yeasts predominate, resulting in accumulation of alcoholic substances and phenolic compounds. In addition, during fermentation, molds like *A. oryzae*, *A. sojae*, *Monilis*, *Penicillium*, and *Rhizopus* may appear on the surface of the mash. However, these molds are believed to have no effects on proper fermentation or aging (16).

To speed up lactic fermentation in the initial stage of soy sauce fermentation, pure cultured lactobacilli are added to the new mash. Similarly, to accelerate the alcoholic fermentation and to shorten its development time, pure cultured yeasts, *Z. rouxii*, are sometimes added to the shoyu mash when its pH value reaches about 5.3, usually three to four weeks after the mash making. The addition of *Torulopsis* yeasts along with *Z. rouxii* is recommended to obtain good volatile flavors.

Kobayashi and Hayashi (17) conducted a study modeling combined effects of factors on the growth of *Z. rouxii* in soy sauce mash. They found that the growth of *Z. rouxii* in soy sauce mash was significantly affected by the pH, temperature, and nitrogen concentration. Furthermore, the pH had an estimated threefold greater influence on the growth of *Z. rouxii* at a nitrogen concentration of 1.5% (wt./vol.) than at 1.0% (wt./vol.)

Color and Flavor Formation

Besides biological and enzymatic reactions, some chemical and physicochemical interactions among the constituents of mash proceed throughout this stage as well as the refining stage. All these complex reactions lead to color and flavor formation of shoyu. For example, during *koikuchi* shoyu brewing, about 50% of its color forms during fermentation and aging stages, and the remaining 50% results from pasteurization. Both are considered to be caused primarily by heat-dependent browning, commonly known as the Maillard browning reaction between amino compounds and sugars, while enzymatic color reactions are rare (5).

The characteristic blackish-purple or blackish-brown color of soy sauce, developed during fermentation, is not always desirable for some applications in which original color should be preserved or other color is more desirable. In this case, color removal or coloration with other colors is necessary. There is a patented method for making colorless or colored soy sauce in the literature (18).

Nearly 300 kinds of volatile components have been identified to date as flavor contributors in *koikuchi* shoyu, and most of these compounds are thought to be generated during brine fermentation. Among them are 37 hydrocarbons, 30 alcohols, 41 esters, 15 aldehydes, 5 pyrones, 25 pyrazines, 7 pyridines, 11 sulfur compounds, 3 thiazoles, 3 terpenes, and 8 other miscellaneous compounds. The most important components of shoyu flavor seem to reside in its weak

acidic fraction, including 4-hydroxyfuranones, many phenolic compounds, such as 4-ethylguaiacol, 4-ethylphenol, 2-phenylethanol, and some alcohols and esters such as maltol, furfural alcohol, and ethyl acetate (5,19–21). When a shoyu is neutralized with alkali, its flavor immediately disappears and does not return in full strength when acidified. In addition, at a lower pH value such as in the range of 4.6–5.0, sensory tests of shoyu flavor yield better ratings (5).

Formation of Sugars and Alcohols

The koji enzymes also convert wheat starch into sugars. Adequate sugar development is important to the finished soy sauce because it subdues the saltiness. Although glucose is the primary sugar, more than 10 others have been isolated. Yeast acts upon a portion of these sugars to form alcohols. Ethanol is the predominant of these and imparts many flavor and aromatic characteristics. It also indicates the presence of other aromatic compounds produced by fermentation. Ethanol content varies depending on the type of soy sauce. In tamari sauce, for example, the lower level of wheat does not contribute enough starch to create ethanol, so its flavor profile is entirely different.

Formation of Amino Acids

During brine fermentation, the proteolytic enzymes in koji play an important role in liberating amino acids from proteins. These amino acids and peptides contribute a full, robust flavor. Among these enzymes, glutaminase is indispensable. This is because glutaminase has an ability to transform glutamine liberated by peptidases from soy protein into glutamic acid, which imparts delicious taste known as *umami* in Japanese. When glutaminase is insufficient or inactivated, glutamine tends to change nonenzymatically into pyroglutamic acid, which is not flavorful compared to glutamic acid. Finished soy sauce contains between 1.5% and 1.65% total nitrogen weight per volume, with glutamic acid being the predominant amino acid.

Kuroshima and colleagues (22) reported that glutamic acid present in the average shoyu on the Japanese market consists of 60% free glutamic acid, 10% pyroglutamic acid, and 30% a conjugated form. They also found that glutaminase is very sensitive to heat, and its activity rapidly decreases in new mash. Shikata and colleagues (23) separated the glutaminase in koji molds into two fractions, water soluble and insoluble. The latter, which remains in the cells, is more resistant to heat and salt and is therefore the major contributor to the production of free glutamic acid. Therefore, adding heat- and salt-resistant glutaminase—produced by some specially bred yeasts—to the new mash is effective in increasing the glutamic acid content of the final product as long as the temperature of the mash is below 60°C (24).

Function of Salt

The brine added at the beginning of fermentation contributes saltiness, with the finished salt concentration ranging from 12% to 18%. But the salt is not there only for

flavor. It is essential to the process. If, for example, the added salt level were reduced, the lactic acid bacteria and yeast in the moromi would act differently and yield a product with a very different flavor profile. The salt concentration is also necessary to help protect the finished sauce from spoilage.

Enzymatic Method—An Alternative to Traditional Fermentation

The traditional methods, either with or without pure culture, all start with transforming raw materials into koji followed by fermenting koji with brine into soy sauce. Such methods are not only complex and laborious, but also lead to losses of nutrients during koji making. To overcome these problems, in recent years, some soy sauce manufacturers have developed a new method using koji enzymes. Soybeans and wheat flour, after proper heat treatment, are first mixed with brine, koji enzymes, and an inoculum. The mixture then undergoes fermentation. After 15 days, the product is ready for packaging. Since the step of koji making is eliminated, labor and cost saving is obvious. Koji enzyme powder is made in a similar way as making koji starter except that the mature koji is finally dried and made into powder. The inoculum contains yeasts and lactic bacteria.

Chemical and Semichemical Soy Sauce

Traditionally, soy sauce is made by fermentation as described. However, soy sauce can also be made by acid hydrolysis. The resulting product is known as chemical soy sauce, nonbrewed soy sauce, or protein chemical hydrolysate. The production of chemical soy sauce is entirely different from that of fermented soy sauce. In brief, defatted soy flour is first hydrolyzed by heating with 18% hydrochloric acid for 15–20 hours. When hydrolysis leads to maximum amount of amino acid production, the mixture is cooled to stop the hydrolytic reaction. Hydrolysate is then neutralized with sodium carbonate, mixed with active carbon, and finally filtered to remove the insoluble materials. Caramel, corn syrup, and salt are typically added to the hydrolysate. Finally, the mixture is refined and packaged. Hydrolysis can also be performed through an enzymatic process with the use of bacterial proteinases (25).

There are several fundamental differences between fermented soy sauce and chemical soy sauce. First, fermented soy sauce has a long history as a human food, whereas chemical soy sauce has a history of only several decades. Second, it takes at least several weeks to make soy sauce by fermentation, most often several months, whereas chemical soy sauce can be made within one day. As a result, the cost to make chemical soy sauce is much lower. And third, in making fermented soy sauce, the proteins and carbohydrates in the raw materials are hydrolyzed slowly under mild conditions by the enzymes of *Aspergillus* species, salt-tolerant yeasts, and lactic bacteria, whereas in chemical soy sauce, they are hydrolyzed quickly with hydrochloric acid.

The last difference in processing mechanisms leads to major differences in chemical composition and organoleptic features between chemical soy sauce and fermented soy sauce (19,26). During chemical hydrolysis, the carbohydrates may be converted into undesirable compounds such as dark humins, levulinic acid, and formic acid, which are not found in fermented soy sauce (8). In addition, some amino acids and sugars produced are destroyed by the acid, resulting in not only imbalance of amino acid profile (particularly the ratio of glutamic acid content to total nitrogen) but also production of undesirable compounds responsible for bad odors and flavors. For example, dimethyl sulfide, hydrogen sulfide, and furfural are derived from methionine, sulfur-containing amino acids, and pentose, respectively, while tryptophan, one of the nutritionally important amino acids, is destroyed almost completely. The differences in major chemical components between brewed and nonbrewed soy sauces are shown in Table 13.1.

Consequently, chemical soy sauce normally does not possess the flavor and odor of fermented soy sauce. To improve its quality, chemical soy sauce is often blended with fermented soy sauce to become a semichemical product before being sold. Alternatively, a semichemical procedure is sometimes used. In this process, soybeans or soy flour is hydrolyzed with a lower concentration of hydrochloric acid. The resulting hydrolyzate is then fermented with osmophilic yeasts in the presence of wheat koji (8,27).

Finally, brewed or fermented soy sauce has a cleaner label. Because soy sauce has no standard of identity in the United States, its contents must be declared as ingredients on its label. For example, for a fermented soy sauce, the ingredient list may look like this: water, wheat, soybean, salt, with or without sodium benzoate as preservative. However, an ingredient list for nonbrewed soy sauce may look like this: water, hydrolyzed corn and soybean protein, corn syrup, salt, citric acid, caramel, and sodium benzoate.

TABLE 13.1

Differences in Chemical Components between Brewed (Fermented) and Nonbrewed (Chemical) Soy Sauces

Component	Unit	Brewed	Nonbrewed
Sodium chloride	g/100 ml	16.00	18.20
Total nitrogen	g/100 ml	1.65	1.29
Amino acid	Total nitrogen	0.49	0.49
Glutamic acid	g/100 ml	1.10	1.28
	Total nitrogen	0.65	1.00
Reducing sugar	g/100 ml	3.00	4.95
Alcohol	g/100 ml	2.40	0.20
Titrateable acidity	g/100 ml	2.20	0.85
Levulinic acid	g/100 ml	0.00	0.61

Data adapted from Anonymous (1).

Aside from the difference in methods to make soy sauce by using soy and wheat material, many imitation soy sauces can be produced with nonsoy materials. These include seafood, mushrooms, and other proteinaceous materials. Otero and colleagues (28) reported an imitation soy sauce made by hydrolyzing dried yeast *Candida utilis* and claimed that it was as good as commercial chemical soy sauce.

Proximate Composition, Quality Attributes, and Grades

The chemical composition in soy sauce is rather complex and varies with types and even batches. According to Yokotsuka (5), in a typical Japanese fermented soy sauce, the soluble solids are divided almost equally between inorganic (46%) and organic (47%) components. Sodium and chlorine are the principal inorganic constituents. Amino acids are the principal organic components, comprising almost 25% of the total soluble solids, followed by carbohydrates, 13%; polyalcohols, 5%; and organic acids, nearly 3%. Of the total nitrogen, about 40–50% are amino acids, 40–50% peptides and peptones, 10–15% ammonia, and less than 1% protein. There are 18 amino acids present and glutamic acid and its salts are the principal flavoring agents. Sugars present are glucose, arabinose, xylose, maltose, and galactose, whereas sugar alcohols are glycerol and mannitol. Organic acids found in shoyu are lactic, acetic, succinic, citric, formic, and pyroglutamic. In addition, there exist trace amounts of organic bases, such as ardenine, hypoxanthine, xanthine, quanine, cytosine, and uracil, all of which are believed to be metabolites of nucleic acids.

In general, a good soy sauce has a salt content of about 18% and a pH value between 4.6 and 4.8. A product with a pH below this range is considered too acidic, suggesting acid production by undesirable bacteria. Other quality factors include nitrogen yield, total soluble nitrogen, and the ratio of amino nitrogen to total soluble nitrogen. The nitrogen yield is the percentage of nitrogen of raw materials converted to soluble nitrogen in the finished product, showing the efficiency of enzymatic conversion. The total soluble nitrogen is a measure of the concentration of nitrogenous material in the shoyu, indicating a standard of quality. The ratio of amino nitrogen to total nitrogen is an accepted standard for overall quality of a soy sauce. The higher the ratio value, the better the quality. The normal range is 50–60%. All these quality attributes are affected by factors related to nearly every step of processing, including raw materials, steaming conditions, tane-koji, and brine fermentation.

As mentioned earlier, in Japan there are five types of soy sauce that are officially recognized. Under each type of soy sauce, the Japanese government assigns three grades based on organoleptic evaluation, total nitrogen content, soluble solids other than sodium chloride, and color. They are Special, Upper, and Standard. Since the quality of chemical soy sauce is generally considered inferior to fermented soy sauce, a soy sauce mixed with semichemical or chemical soy sauce cannot be graded as Special. In other words, Special grade is assigned to high quality, brewed soy sauce only.

In the middle of the 1960s, the possible presence of aflatoxins in soy sauce and other fermented products that use koji was raised as a concern, because the main mold, *Aspergillus flavus*, which produces carcinogenic aflatoxins in peanuts, corn, and a few other foods when not stored properly, is a close relative of *Aspergillus oryzae*, the main mold in koji. However, after extensive surveys and tests, it is concluded that none of the koji strains produce aflatoxins (29) or such weak toxic mycotoxins as aspergillilic acid, kojic acid, β -nitropropionic acid, oxalic acid, and formic acid (30). Most recently, Matsushima and colleagues (31) showed that the absence of aflatoxin biosynthesis in koji molds is due to a defect in af1R gene expression. Therefore, soy sauce is safe to consume.

Application of Soy Sauce

As an all-purpose seasoning, soy sauce offers a wide range of applications. Soy sauce not only contributes a unique flavor profile to traditional Asian foods but also holds great potential as a flavoring and flavor-enhancing material for a wide variety of non-Asian food products. The key factor for success is to determine the optimal level of use. This will vary depending on the product and the desired effect. If used at too high a level, soy sauce can produce bitter, off-flavor. [Table 13.2](#) lists what soy sauce can do as a flavoring to virtually every category of Western food.

Soy sauce contributes functional benefits to processed food. Although soy sauce cannot act as the sole preservative, its acid, alcohol, and salt content contribute to the overall preservative effect. Its lactic acid content also allows soy sauce to function as an acidulant in foods, such as bean dip, in which a harsh acid bite would be undesirable. Furthermore, many of its components also contribute a strong antioxidant effect when applied to food. Long and colleagues (2) compared the total antioxidant activities of several seasonings in Asian cooking and found that dark soy sauce has a powerful antioxidant activity. Chiou and colleagues (3) reported that soy sauce protected ground pork-fat patties from oxidation. Soy sauce has also been shown to have antiplatelet activity (4). Therefore, it possesses possible health benefits for the body and may be considered a functional seasoning.

Besides contributing directly to flavor and functionality, soy sauce is a natural flavor enhancer and can serve as an alternative to glutamate (32). The key components are amino acids. Many amino acids have been identified both as flavor potentiators and as umami contributors—most notably, glutamic acid. Umami is the fifth flavor, coined by the Japanese, in addition to the well-recognized four basic flavors—sweet, salty, sour, and bitter. Often translated as “savory” or “brothy,” umami can be described as the tongue-coating, meaty flavor of sautéed mushrooms, a juicy steak, or a rich stock. Umami ingredients, such as glutamic acid, may work synergistically with salt to produce an enhancing effect. Thus, adding brewed soy sauce to a variety of food products can help achieve this elusive fifth flavor, making foods taste richer and more fully rounded (33).

TABLE 13.2**Applications of Soy Sauce on Various Types of Food**

Food Products	Functions that Soy Sauce Fulfills
Bacon and cured meats	Add color, balance sweet and smoked flavor, contribute salt for curing, and add natural preservatives.
Beef and beef entrees	Contribute savory flavor, add color, help blend spice flavor, and enhance aroma.
Bread and rolls	Contribute salt to moderate yeast activity, help blend yeast and grain flavor notes, add color.
Chicken and chicken entrees	Contribute savory flavor, help blend spice flavors, enhance aroma.
Chocolate syrups and coating	Blend dairy notes, sweetness and cocoa flavor, moderate sweetness, enhance fruity top notes (of flavor), contribute color.
Cookies and cakes	Help blend flavors and add complexity, temper sweetness, add color, enhance fruity top notes of chocolate chips, if any.
Dry mixes	Add savory notes, enhance aroma and flavor for homemade appeal, granulated forms dissolve easily when prepared in the home, contribute color.
Fajitas and Mexican entrees	Blend and enhance spices in marinade, contribute salt, helps enhance grilled color, enhance meaty flavor in quick-grilled application.
Gingerbread	Add color, help blend spice flavors, moderate sweetness.
Jerky	Contribute salt for curing, blend spice flavors, enhance meaty flavors, contribute color, can enhance or even replace preservatives.
Pasta salad	Smooth the harshness of vinegar, blend and enhance spice flavors, contribute salt.
Salad dressings	Add savory flavor, help temper vinegar's harshness, help condiments, blend spice flavors, contribute preservation to cold-filled dressings, add color, and replace Worcestershire sauce.
Snack	Blend flavors of other seasoning ingredients, contribute salt, add color, provide savory flavor.
Soups, stew, broths	Enhance overall flavor profile, contribute aroma, and add color.

Adapted from Anonymous (1).

There are certain applications in which it is best not to use soy sauce. If a food is already rather sweet, salty, or sour, the addition of soy sauce should be approached with caution. For example, the salt of soy sauce may be incompatible with dominant sweet or sour tastes, or its acid level may simply make the product entirely too tart. Soy sauce should not be used for foods created for sodium-restricted diets since even reduced-salt versions still contain a significant amount of salt.

References

1. Anonymous, *The Soy Sauce Handbook: A Reference Guide for Food Manufacturers*, Kikkoman International Inc., San Francisco, California, 2000.
2. Long, L.H., D. Chua, T. Kwee, and B. Halliwell, The Antioxidant Activities of Seasonings Used in Asian Cooking: Powerful Antioxidant Activity of Dark Soy Sauce Revealed Using the ABTS, *Free Radic. Res.* 32:181–186 (2000).

3. Chiou, R.Y.Y., K.L. Ku, L.S. Lai, and L.G. Chang, Antioxidative Characteristics of Oils in Ground Pork-Fat Patties Cooked with Soy Sauce, *J. Am. Oil Chem. Soc.* 78:7–11 (2001).
4. Tsuchiya, H., M. Sato, and I. Watanabe, Antiplatelet Activity of Soy Sauce as Functional Seasoning, *J. Agric. Food Chem.* 47:4167–4174 (1999).
5. Yokotsuka, T., Soy Sauce Biochemistry, *Adv. Food Res.* 30:196–329 (1986).
6. Liu, K.L., *Soybeans: Chemistry, Technology, and Utilization*, Aspen Publishers, Inc., Gaithersburg, Maryland, 1999.
7. Huang, T.-C., and D.-F. Teng, Soy Sauce: Manufacturing and Biochemical Changes, Chap. 29 in *Handbook of Food and Beverage Fermentation Technology*, edited by Y.H. Hui, L. Meunier-Goddik, A.S. Hansen, J. Josephsen, W-K Nip, P.S. Stanfield, and F. Toldra, Marcel Dekker, New York, 2004, pp. 497–532.
8. Fukushima, D., Fermented Vegetable (Soybean) Protein and Related Foods of Japan and China, *J. Am. Oil Chem. Soc.* 56:357–362 (1979).
9. Watanabe, T., and A. Kishi, *Nature's Miracle Protein: The Book of Soybeans*, Japanese Publications, Inc., Tokyo, 1984.
10. Kinoshita, E., T. Sugimoto, Y. Ozawa, and T. Aishima, Differentiation of Soy Sauce Produced from Whole Soybeans and Defatted Soybeans by Pattern Recognition Analysis of HPLC Profiles, *J. Agric. Food Chem.* 46:977–883 (1998).
11. Chou, C.C., and M.Y. Ling, Biochemical Changes in Soy Sauce Prepared with Extruded and Traditional Raw Materials, *Food Res. Int.* 31:487–482 (1998).
12. Kikuchi, T., H. Sugimoto, and T. Yokotsuka, Polysaccharides in Pressed Cake and Their Effects on Difficulty in Press Filtration of Fermented Soy Sauce Mash, *J. Agric. Chem. Soc. Jpn.* 50:279–286 (1976).
13. Hashimoto, H., and T. Yokotsuka, Mechanisms of Sediment Formation During Heating of Raw Shoyu, *J. Brew. Soc. Jpn.* 71:496–499 (1979).
14. Fukushima, D., Soy Proteins for Foods Centering Around Soy Sauce and Tofu, *J. Am. Oil Chem. Soc.* 58:346 (1981).
15. Komatsu, Y., Changes of Some Enzyme Activities in Shoyu Brewing. 1. Changes of the Constituents and Enzymes Activities in Shoyu Fermentation after Low-Temperature Mashing, *Seasoning Sci. (Jpn.)* 15:10–20 (1968).
16. Yokotsuka, T., Aroma and Flavor of Japanese Soy Sauce, *Adv. Food Res.* 10:75–134 (1960).
17. Kobayashi, M., and S. Hayashi, Modeling Combined Effects of Temperature and pH on the Growth of *Zygosaccharomyces rouxii* in Soy Sauce Mash, *J. Ferment. Bioeng.* 85:638–641 (1998).
18. Tokita, H., I. Matsui, H. Hasegawa, S. Taima, K. Ohyoshi, H. Sugita, *et al.*, Colored Shoyu (Soy Sauce), U.S. Patent, 5,030,461, July 9, 1991.
19. Nunomura, N.N., M. Sasaki, Y. Asao, and T. Yokotsuka, Identification of Volatile Components in Shoyu (Soy Sauce) by Gas Chromatography, *Agric. Biol. Chem.* 40:485–490 (1976).
20. Nunomura, N.N., M. Sasaki, and T. Yokotsuka, Shoyu (Soy Sauce) Flavor Components: Acetic Fractions and the Characteristic Flavor Component, *Agric. Biol. Chem.* 44:339–351 (1980).
21. Yong, F.M., K.H. Lee, and H.A. Wong, The Production of Ethyl Acetate by Soy Yeast (*Saccharomyces rouxii* Y-1096), *J. Food Technol.* 16:177 (1981).
22. Kuroshima, E., Y. Oyama, T. Matsuo, and T. Sugimori, Biosynthesis and Degradation of Glutamic Acid in Microorganisms Relating to the Soy Sauce Brewing. (III). Some

- Factors Affecting the Glutamic Acid and its Related Substances Formation in Soy Sauce Brewing, *J. Ferment. Technol.* 47:693–700 (1969).
23. Shikata, H., T. Yasui, U. Ishigami, and K. Omori, Studies on the Glutaminase of Shoyu Koji (Part I), *J. Jpn. Soy Sauce Res. Inst.* 4:48–52 (1978).
 24. Yokotsuka, T., T. Iwasa, S. Fujii, and T. Kakinuma, The Role of Glutaminase in Shoyu Brewing. Annual Meeting of the Agricultural Chemistry Society of Japan, April 1, 1972, Sendai, Japan.
 25. Olsen, H.A.S., Method of Producing Soy Protein Hydrolysate from Fat-Containing Soy Material and Soy Protein Hydrolysate, U.S. Patent 4,324,805, April 13, 1982.
 26. Uchida, K., Trends in Preparation and Uses of Fermented and Acid-Hydrolyzed Soy Sauce, in *Proceedings of the World Congress: Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, edited by T.H. Applewhite, American Oil Chemists' Society, Champaign, Illinois, 1989.
 27. Tenbata, M., and T. Morinaga, Fermenting Ability and the Refined Degree of Soy Moromi by Addition of Chemical Soy Sauce, *Hiroshima-ken Shokuhin Kogyo Shikensho Hokoku (Jpn.)* 10:37–44 (1968).
 28. Otero, M.A., A.J. Cabello, M.C. Vasallo, L. Garcia, and J.C. Lopez, Preparation of an Imitation Soy Sauce from Hydrolyzed Dried Yeast *Candida utilis*, *J. Food Proc. Pres.* 22:419–432 (1998).
 29. Hesseltine, C.W., O.L. Shotwell, J.J. Ellis, and R.D. Stubblefield, Alfatoxin Formation by *Aspergillus flavus*, *Bacteriol. Rev.* 30:795–805 (1966).
 30. Yokotsuka, T., K. Oshita, T. Kikuchi, and M. Sasaki, Studies on the Compounds Produced by Molds. VI. Aspergilllic Acid, Koji Acid, β -Nitropropionic Acid, and Oxalic Acid in Solid-Koji, *J. Agric. Chem. Soc. Jpn.* 43:189–196 (1969).
 31. Matsushima, K., K. Yashiro, Y. Hanya, K. Abe, K. Yabe, and T. Hamasaki, Absence of Aflatoxin Biosynthesis in Koji Mold (*Aspergillus sojae*), *Appl. Microbiol. Biotechnol.* 55:771–776 (2001).
 32. Eber, M., and W.D. Muller, Spray Dried Soy Sauce as Flavor Enhancer—Alternative or Competition to Glutamate? *Fleischwirtschaft* 78:1276–1277 (1998).
 33. Yoshida, Y., Umami Taste and Traditional Seasoning, *Food Rev. Int.* 14:213–246 (1998).

Chapter 14

Breeding Specialty Soybeans for Traditional and New Soyfoods

Zhanglin Cui^a, A.T. James^b, Shoji Miyazaki^c, Richard F. Wilson^d, and Thomas E. Carter Jr.^e

^aNorth Carolina State University, Raleigh, NC 27607; ^bCSIRO Division of Plant Industries, Indooroopilly, Australia 4068; ^cNational Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan; ^dUnited States Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705; ^eUnited States Department of Agriculture, Agricultural Research Service, Raleigh, NC 27607

Soyfoods (foods made from soybean) have been a part of daily life in Asia for over 5,000 years. This long relationship with soyfoods is one of mankind's most enduring love affairs. Ancient Chinese writings tell us that the affair began modestly enough as a mere flirtation, when inventive cooks first dished up soup made from young green leaves. Although we no longer eat the soybean's leaves today, our relationship has blossomed to embrace literally hundreds of other soy dishes that now delight our palate. The diversity of soyfoods in the human diet is a tribute to humankind's remarkable passion for food. Through trial and error, and continual refinement, perhaps 200 generations of Asian families strove to bring out the best from the soybean and in so doing contributed their much-appreciated recipes to the world's soyfoods repertoire. Tofu, *natto*, *maodou* (*edamame*), soymilk, soy sauce, and soy sprouts are but a few examples.

It should come as no surprise that the age-old human endeavor to create new and better soyfoods has also dramatically altered the essential ingredient of soyfoods—the bean itself. Ancient families possessed keen eyes and palates and did much to create the better bean. It was they who noticed and saved “sports” (spontaneous changes in soybean) that produced a tastier dish or perhaps a more bountiful harvest. Handing these treasures down, parent to child, and fine-tuning family recipes along the way, as many as 40,000 of these sports had been selected in Asia by 1900. Also called landraces (cultivars developed by farmers), they carried many new and desirable genes not found in the original bean. Fortunately, many of these traditional landraces have been preserved in agricultural germplasm banks, and today are used as genetic resources to further improve the soyfoods that we love to eat.

This chapter summarizes the history and current status of the breeding of soyfoods and other specialty cultivars in the United States, China, Australia, and Japan. Recent advances in food technology have given rise to novel soyfoods, such as soy ice cream, soy burgers and hot dogs, soy-substitute chicken nuggets, and soy-based baby foods. Current work on genetic adaptation of soybean for these new uses is also reviewed in this chap-

ter. In addition, this chapter reviews factors and traits that determine current breeding strategy for various soyfoods markets, and suggests new avenues for designing soyfoods cultivars with improved seed composition. This review also provides a detailed list of modern, publicly released, soyfoods cultivars on a country-by-country basis.

Soybean and Soyfoods in China

Domestication of Soybean

It is commonly believed that cultivated soybean (*Glycine max* L. Merr.) was domesticated from wild soybean (*Glycine soja* Seib. et Zucc.) in ancient China perhaps 3,000 to 5,000 years ago (1,2). This estimate is derived, in part, from references to soybean that appeared in Chinese literature almost as soon as written characters were developed, during the Shang dynasty (1700 BC to 1100 BC) (3). Proverbs and other oral traditions recorded during that time indicate the importance of soybean in daily life and suggest a much older association of soybean with Chinese culture (2). Soybean is believed to have arrived in Japan about 1 AD and in the West before 1765 (4).

Ancient Utilization and Processing

In ancient China, soybean was a staple food crop and a valuable component of medicine, food, and feed (2). Poems from 600 BC to 300 BC mention soup made from young green leaves and stew made from soybean seeds as important meals in China. Soybeans and chicken were described as the major daily food for an emperor from this period (2). Archeologists have confirmed that the tofu making process was invented in the Han dynasty (206 BC–220 AD). A detailed description of tofu processing can be found in the famous ancient Chinese book of medicine, *Ben cao gang mu*, by Li Shizheng (1578 AD) (2). *Douchi*, a fermented salty garnish made from whole soybeans, was produced 2,000 years ago. The processing procedures for *douchi* and *doujiang* (a thick sauce made from fermented soybeans) were described in an ancient Chinese agriculture book, *Qi min yao shu* (630 AD). The history of soybean oil processing can be traced back at least 1,000 years, when Chinese people fried tofu with soy oil to make a tasty dish (2). The use of soybean as a green vegetable (*maodou* in Chinese) was first recorded about 1000 AD. *Maodou* as a specific term first appeared in literature from the Ming dynasty during the 17th century. At that time, roasted or boiled green vegetable soybeans were eaten as a snack. Many soyfoods were available in local markets as early as the 13th century, including stems covered with green pods, sprouts, soybean biscuits, soybean porridge, and “soybean balls” (2).

Traditional Soyfoods Cultivars

The center of domestication for soybean is believed to be central or southern China. As soyfoods became popular in the diet, farmers practiced genetic selection as they grew the crop, by saving seed from desirable plants and sowing them in the following year (1,3). Over millennia, this process helped to genetically adapt soybean for myriad soyfood uses, facilitated the spread of the crop across Asia, and integrated soybean

into Chinese culture. Distinct soybean landraces were reported by 1116 AD, when Chinese authors recorded the comparison of green-, brown-, and black-seedcoated, and large- and small-seeded soybean types (2). As many as 40,000 landraces may have been grown in China at the beginning of the 20th century (5,6). Most of these landraces were used in soyfood preparation and many were named for their food use. Examples are *da qin dou* or *da lu dou* (big green beans), *cai dou* (vegetable bean), *mao dou* (hairy pod bean, a desirable trait associated with green vegetables), *dou fu dou* (tofu bean), *dou ya dou* (sprout bean), *xiao li dou* (small-seeded bean for sprouting), *you dou* (oil bean), *yao dou* (medicine bean), and *dou chi dou* (douchi bean).

Current Soyfoods Markets

Today, soybean constitutes one of the most important crops in China. It is the fourth main food crop in both acreage and tonnage after rice, wheat, and corn (7,8). Most of the Chinese soybean production is used in the making of traditional and modern high-protein soyfoods such as various kinds of bean curd (tofu), soymilk, soy ice cream, and textured protein products. Although soymilk has been a traditional popular drink in the Chinese home, it has only recently become a popular item in the marketplace, in part the result of improved preservation and packaging techniques employed in the emerging soymilk industry. Edible oil is the second most important food product derived from soybean, after the aforementioned high-protein soyfoods. Soy sauce and other fermented products (such as *douchi*) are probably the third most important category of soy products. Soybean continues to be consumed as sprouts, as a fresh vegetable, and as medicine and is grown on a relatively small scale for these purposes. Small-seeded soybeans are exported to Japan for natto processing.

Very little soybean was imported into China for traditional soyfoods preparation before 1990. However, soybean importation into China from 1990 to 1996 increased from 1,000 tons to 1.1 million tons. Exports dropped from 940,000 tons to 190,000 tons during this same period (8). This shift resulted in part from an increase in soyfoods consumption driven by new soyfoods processing businesses and by government-sponsored health-action plans that promoted the drinking of soybean milk in elementary, middle, and high schools.

Modern Soyfoods Cultivars

Modern soybean breeding emerged in China as early as 1913 with the establishment of the first soybean breeding institution at Gongzhuling Agricultural Experiment Station (now Jilin Academy of Agricultural Sciences) in the northeast (9). Professor Shou Wang released the first improved soybean cultivar “Jin da 332” for the lower Changjiang (Yangtze) valley in 1923. Manual cross-pollination was first employed in 1927. The first cultivar from hybridization, Man Cang Jin, was developed in 1935 and released in 1941. Man Cang Jin became an important parent in subsequent Chinese and Japanese breeding. By 1995, modern breeding efforts had led to the release of 651 public cultivars in China (10,11). Although most modern Chinese cultivars are crushed for meal and oil, 193 of these modern cultivars were released specifically for the soy-

foods market (10,11) Tables 14.1 and 14.2 provide details of the region of origin, date of release, and specialty traits of these 193 cultivars. Seed appearance and composition are determining factors for the selection of cultivars for specific soyfoods applications.

Cultivars for Bean Curd (Tofu) and Soymilk. Although genetic differences in tofu yield and quality has long been known among soybean landraces and cultivars in China, systematic genetic research on tofu began only in the 1970s. Tofu and soymilk processing traits have become important breeding objectives since 1980. Breeding for improved tofu yield became a national objective in the Chinese National Soybean Breeding Program in 1986. Several recent reports document useful genotypic variation and inheritance of tofu yield and quality traits related to soybean landraces in China (12–15). Several new soyfoods cultivars, such as Uspqo-2 and Qian do 4 Hao, with high tofu yield have been developed (Table 14.2). Recently, one landrace imparting a fragrant aroma to fresh tofu was discovered (12).

Cultivars for Small-Seeded Soybeans (Sprouts, Natto). Fresh bean sprouts are a traditional vegetable in China. Small-seeded types (100-seed weight of 10–15 g) are generally used for sprout production. A large number of traditional landraces and modern cultivars satisfy this requirement. As a result, there has been little systematic breeding effort to develop improved cultivars for sprouts in China. However,

TABLE 14.1
Distribution of Releases of 193 Public Soyfood Cultivars Developed in China from 1923 to 1995

Primary specialty trait ^a	Release era						Region			
	20s–40s	50s	60s	70s	80s	90s	North-east	North	South	Total ^b
High protein	0	1	2	6	27	21	6	17	34	57
Vegetable	0	0	2	12	20	19	6	14	33	53
High protein and oil	8	3	2	6	16	9	26	9	9	44
High oil	0	0	10	12	10	2	28	4	2	34
Large seed	0	0	2	4	12	6	5	6	13	24
Small seed	1	0	1	3	9	2	6	5	5	16
Tofu	1	1	0	1	3	1	1	0	6	7
Natto	0	0	0	0	5	2	6	1	0	7
Douchi	0	1	0	2	1	1	0	1	4	5
Medicine	0	0	0	1	3	1	0	3	2	5
Soy sauce	1	0	0	2	0	0	1	0	2	3
Total ^b	9	5	18	40	74	47	75	48	70	193

^aHigh protein, protein content >45%; high oil, oil content >23%; high protein and oil, total content of protein and oil >63%; vegetable, released for *maodou* use (immature green soybean seed); *douchi*, suitable for making the fermented and salted soybean food; *natto*, small-seeded type for export to Japan; small seed, suitable for *natto* (100-seed weight <12 g) or sprout (100-seed weight 10–15 g); large seed, suitable for *maodou* or *tofu* (100 seed weight >25 g).

^bSome cultivars fit more than one category of specialty trait; totals refer to number of unique cultivars developed during a specified release era or released from a specific region.

TABLE 14.2

Origin and Description of 193 Soyfood Cultivars Released in China from 1923 to 1995

Cultivar name ^a	Province of origin	Year of release	Specialty trait(s) ^b
58-161	Jiangsu	1964	High protein
7605	Shandong	1986	<i>Natto</i>
Ai Jiao Qing	Jiangxi	1974	Vegetable, large seed
An Dou 1 Hao	Guizhou	1988	High protein
An Dou 2 Hao	Guizhou	1988	Small seed, high protein
Ba Hong 1 Hao	Hebei	1972	Vegetable, small seed
Bei Feng 2 Hao	Heilongjiang	1983	High oil
Bo Xian Da Dou	Anhui	1975	Vegetable, large seed
Chang Bai 1 Hao	Jilin	1982	<i>Natto</i>
Cheng Dou 4 Hao	Sichuan	1989	Vegetable
Cheng Dou 5 Hao	Sichuan	1993	High protein
Chu Xiu	Jiangsu	1992	Vegetable, large seed
Chuan Dou 2 Hao	Sichuan	1993	High protein
Dan Dou 1 Hao	Liaoning	1970	Vegetable
Dan Dou 3 Hao	Liaoning	1975	High oil
Dan Dou 4 Hao	Liaoning	1979	Vegetable
Dan Dou 6 Hao	Liaoning	1989	Vegetable, large seed
Dong 2	Guizhou	1988	High protein
Dong Mu Xiao Li Dou	Heilongjiang	1988	<i>Natto</i>
Dong Nong 36	Heilongjiang	1983	High protein
Dong Nong 37	Heilongjiang	1984	High PO
Dong Nong 40	Heilongjiang	1991	Vegetable
Dong Nong Chao	Heilongjiang	1993	<i>Natto</i>
Xiao Li 1 Hao			
E Dou 4 Hao	Hubei	1989	High protein
Feng Xi 1 Hao	Liaoning	1960	Large seed
Feng Xi 12	Liaoning	1965	Vegetable
Feng Xi 2 Hao	Liaoning	1960	Large seed
Feng Xi 6 Hao	Liaoning	1965	High PO
Gan Dou 1 Hao	Jiangxi	1987	High protein
Gan Dou 2 Hao	Jiangxi	1990	Vegetable, large seed, high protein
Gong Dou 2 Hao	Sichuan	1990	Vegetable
Gong Dou 3 Hao	Sichuan	1992	Vegetable
Gong Dou 7 Hao	Sichuan	1993	Vegetable
Gong Jiao 5601-1	Jilin	1970	High oil
Gong Jiao 5610-1	Jilin	1970	High oil
Gong Jiao 5610-2	Jilin	1970	High oil
Guan Yun 1 Hao	Jiangsu	1974	High protein
He Jiao 13	Heilongjiang	1968	High oil
He Jiao 6 Hao	Heilongjiang	1963	High oil
He Nan Zao Feng 1 Hao	Henan	1971	Small seed
Hei Nong 16	Heilongjiang	1970	High oil
Hei Nong18	Heilongjiang	1970	High PO
Hei Nong 27	Heilongjiang	1983	High PO
Hei Nong 31	Heilongjiang	1987	High oil
Hei Nong 32	Heilongjiang	1987	High oil
Hei Nong 4 Hao	Heilongjiang	1966	High oil

Cultivar name ^a	Province of origin	Year of release	Specialty trait(s) ^b
Hei Nong 6 Hao	Heilongjiang	1967	High oil
Hei Nong 8 Hao	Heilongjiang	1967	High oil
Hei Nong Xiao Li	Heilongjiang	1989	<i>Natto</i>
Dou 1 Hao			
Hong Feng 3 Hao	Heilongjiang	1981	High oil
Hong Feng 9 Hao	Heilongjiang	1995	High oil
Hong Feng Xiao Li	Heilongjiang	1988	<i>Natto</i>
Dou 1 Hao			
Hua 75-1	Henan	1990	Large seed
Hua Yu 1 Hao	Henan	1974	Vegetable
Huai Dou 2 Hao	Jiangsu	1986	High protein
Huang Bao Zhu	Jilin	1923	Tofu, soy sauce, high PO
Hui An Hua Mian Dou	Fujian	1958	<i>Douchi</i> , tofu
Ji Dou 4 Hao	Hebei	1984	High PO
Ji Dou 9 Hao	Hebei	1994	Vegetable
Ji Lin 1 Hao	Jilin	1963	High oil
Ji Lin 10 Hao	Jilin	1971	High PO
Ji Lin 12	Jilin	1971	High oil
Ji Lin 14	Jilin	1978	High PO
Ji Lin 24	Jilin	1990	High PO
Ji Lin 28	Jilin	1991	High protein
Ji Lin 6 Hao	Jilin	1963	High oil
Ji Lin 9 Hao	Jilin	1971	High PO
Ji Lin Xiao Li 1 Hao	Jilin	1990	<i>Natto</i>
Ji Qing 1 Hao	Jilin	1991	Vegetable
Ji Ti 4 Hao	Jilin	1956	High PO
Ji Ti 5 Hao	Jilin	1956	High PO
Jian Feng 1 Hao	Heilongjiang	1987	Large seed
Jian Guo 1 Hao	Henan	1977	High protein
Jin Da 36	Shanxi	1989	Large seed
Jin Dou 3 Hao	Shanxi	1974	Vegetable
Jin Dou 514	Shanxi	1978	Vegetable
Jin Dou 7 Hao	Shanxi	1987	Medicine
Jin Dou 8 Hao	Shanxi	1987	Large seed
Jin Jiang Da Li Huang	Fujian	1970	<i>Douchi</i> , soy sauce, tofu
Jin Jiang Da Qing Ren	Fujian	1977	<i>Douchi</i> , soy sauce, medicine
Jin Ning Da Huang Dou	Yunnan	1987	Vegetable
Jin Yuan 2 Hao	Heilongjiang	1941	High PO
Jiu Feng 2 Hao	Heilongjiang	1984	High oil
Jiu Nong 12	Jilin	1982	High PO
Jiu Nong 14	Jilin	1985	Large seed, high PO
Jiu Nong 18	Jilin	1991	High PO
Jiu Nong 4 Hao	Jilin	1969	High protein
Ju Xuan 23	Shandong	1963	Small seed
Kai Yu 10 Hao	Liaoning	1989	High PO
Ke Xi 283	Heilongjiang	1956	High PO
Ke Xin 3 Hao	Beijing	1995	High protein
Ken Nong 4 Hao	Heilongjiang	1992	High PO
Li Qiu 1 Hao	Zhejiang	1995	High protein

(Continued)

TABLE 14.2*(Cont.)*

Cultivar name ^a	Province of origin	Year of release	Specialty trait(s) ^b
Liang Dou 2 Hao	Sichuan	1986	Vegetable, high PO
Lin Dou 3 Hao	Shandong	1975	Small seed, high PO
Ling Dou 1 Hao	Anhui	1977	High protein
Liu Shi Ri	Jiangsu	1973	High protein
Lu Bao Zhu	Jiangsu	1992	Vegetable, large seed
Lu Dou 10 Hao	Shandong	1993	High protein
Lu Dou 2 Hao	Shandong	1981	High PO
Lu Hei Dou 1 Hao	Shandong	1992	Vegetable, <i>douchi</i> , medicine
Lu Hei Dou 2 Hao	Shandong	1993	Vegetable
Mao Peng Qing 1 Hao	Zhejiang	1988	Vegetable, tofu, high protein
Mao Peng Qing 2 Hao	Zhejiang	1988	Vegetable, tofu, large seed, high protein, high PO
Mao Peng Qing 3 Hao	Zhejiang	1988	Vegetable, large seed
Meng Qing 6 Hao	Anhui	1974	Vegetable, large seed
Mu Feng 1 Hao	Heilongjiang	1968	High oil
Nan Nong 87C-38	Jiangsu	1990	Vegetable, high protein
Nan Nong Cai Dou 1 Hao	Jiangsu	1989	Vegetable, large seed
Nen Feng 1 Hao	Heilongjiang	1972	High oil
Nen Feng 10 Hao	Heilongjiang	1981	High oil
Nen Feng 13	Heilongjiang	1987	High PO
Nen Feng 2 Hao	Heilongjiang	1972	High oil
Nen Feng 4 Hao	Heilongjiang	1975	High oil
Nen Feng 7 Hao	Heilongjiang	1970	High oil
Ning Qing Dou 1 Hao	Jiangsu	1987	Vegetable, high protein
Ning Zhen 1 Hao	Jiangsu	1984	Vegetable
Ning Zhen 2 Hao	Jiangsu	1990	High PO
Qi Cha Dou 1 Hao	Shandong	1995	Vegetable
Qi Huang 21	Shandong	1979	High oil
Qi Huang 4 Hao	Shandong	1965	High PO
Qian Dou 4 Hao	Guizhou	1995	Tofu, high protein
Qian Jin 2 Hao	Hebei	1976	Vegetable
Qin Jian 6 Hao	Henan	1977	High protein
Shang Qiu 64-0	Henan	1983	Vegetable, large seed, high protein
Shang Qiu 7608	Henan	1980	High protein
Shen Nong 25104	Liaoning	1979	High PO
Sheng Lian Zao	Guizhou	1975	High protein
Su Nei Qing 2 Hao	Jiangsu	1990	Vegetable, large seed
Su Xian 647	Anhui	1925	Small seed
Su Xie 19-15	Jiangsu	1981	Large seed
Sui Nong 3 Hao	Heilongjiang	1973	High oil
Sui Nong 6 Hao	Heilongjiang	1985	High oil
Tai Chun 1 Hao	Jiangsu	1992	Vegetable
Tai Gu Zao	Shanx	1960	High oil
Tie Feng 22	Liaoning	1986	High oil

Cultivar name ^a	Province of origin	Year of release	Specialty trait(s) ^b
Tie Jia Qing	Hebei	1971	Vegetable
Ting Dou 1 Hao	Fujian	1985	Vegetable
Tong Hei 11	Guangdong	1986	Vegetable, small seed, high protein
Tong Nong 10 Hao	Jilin	1992	High protein
Tong Nong 11	Jilin	1995	High protein
Wan Dou 1 Hao	Anhui	1983	High PO
Wan Dou 10 Hao	Anhui	1991	High protein
Wan Dou 3 Hao	Anhui	1984	High PO
Wan Dou 4 Hao	Anhui	1986	High protein
Wu Dou 1 Hao	Neimenggu	1989	High oil, high PO
Xi Bi Wa	Heilongjiang	1941	High PO
Xi Dou 1 Hao	Henan	1980	Vegetable
Xia Dou 75	Jiangsu	1975	Vegetable
Xiang B68	Hunan	1984	<i>Douchi</i> , medicine, small seed
Xiang Chun Dou 11	Hunan	1987	High PO
Xiang Chun Dou 12	Hunan	1989	High oil
Xiang Chun Dou 13	Hunan	1989	Vegetable
Xiang Chun Dou 14	Hunan	1992	High oil
Xiang Chun Dou 15	Hunan	1995	Vegetable, high PO
Xiang Dou 6 Hao	Hunan	1981	Small seed
Xiang Qing	Hunan	1988	Vegetable, high protein
Xiang Qiu Dou 2 Hao	Hunan	1982	Large seed
Xin Liu Qing	Anhui	1991	Vegetable, large seed, high protein, high PO
Xu Dou 135	Jiangsu	1983	High PO
Yan Qing	Fujian	1985	Vegetable, high protein
Yin Huang 3 Hao	Shandong	1985	High protein
You Bian 30	Beijing	1983	High PO
You Chu 4 Hao	Beijing	1994	High protein
Yu Dou 10 Hao	Henan	1989	High protein
Yu Dou 12	Henan	1992	High protein
Yu Dou 16	Henan	1994	High protein
Yu Dou 19	Henan	1995	High protein
Yu Dou 2 Hao	Henan	1985	Large seed, high protein
Yu Dou 4 Hao	Henan	1987	Medicine, vegetable, high protein
Yu Dou 7 Hao	Henan	1988	High protein
Yuan Bao Jin	Heilongjiang	1941	High PO
Zao Chun 1 Hao	Hubei	1994	Vegetable, high protein
Zao Shu 18	Beijing	1992	High PO
Zao Xiao Bai Mei	Liaoning	1950	High protein
Zhe Chun 1 Hao	Zhejiang	1987	Vegetable, high protein
Zhe Chun 2 Hao	Zhejiang	1987	Tofu
Zhe Chun 3 Hao	Zhejiang	1994	Vegetable, high protein
Zhe Jiang 28-22	Zhejiang	1982	Vegetable, high protein
Zheng 104	Henan	1986	High protein
Zhong Dou 14	Hubei	1987	High protein
Zhong Dou 24	Hubei	1989	High protein

(Continued)

TABLE 14.2*(Cont.)*

Cultivar name ^a	Province of origin	Year of release	Specialty trait(s) ^b
Zhong Dou 8 Hao	Hubei	1993	High protein
Zhong Huang 2 Hao	Beijing	1990	High PO
Zhong Huang 3 Hao	Beijing	1990	High PO
Zhong Huang 7 Hao	Beijing	1993	High protein
Zhou Dou 30	Hubei	1987	High protein
Zhuang Yuan Qing Hei Dou	Hebei	1960	Vegetable, high oil
Zi Hua 1 Hao	Jilin	1941	High PO
Zi Hua 2 Hao	Heilongjiang	1941	High PO
Zi Hua 3 Hao	Heilongjiang	1941	High PO
Zi Hua 4 Hao	Heilongjiang	1941	High PO
Zi Jie Dou 75	Shanxi	1977	Large seed

^aSource: Cui *et al.*, 1999 (11).^bHigh protein, protein content >45%; high oil, oil content >23%; high PO, seed oil content >21%, protein content >42%, and total protein and oil content >63%; vegetable, developed specifically for use as *maodou* (immature green soybean seed); *douchi*, suitable for making the fermented and salted soybean food; *natto*, small-seeded type developed specifically for export to Japan (100-seed weight <12 g); small seed, suitable for *natto* (100-seed weight <12 g) or sprout (100-seed weight 10–15 g); large seed, suitable for *maodou* or *tofu* (100 seed weight >25 g).

considerable effort has been devoted to small-seeded soybeans for the Japanese natto market. Seven natto cultivars were released in northeastern and northern China by 1995 (Table 14.2). Both wild soybean accessions and small-seeded landraces were used as a source of the small-seeded trait in natto breeding.

Cultivars for Vegetable Soybeans (Maodou). Vegetable cultivars are usually large-seeded (mature 100-seed weight greater than 25 g). Unlike natto and tofu cultivars, seed coats with green, brown, or black colors are common among vegetable soybean cultivars. References to the immature green vegetable bean as medicine can be found in ancient Chinese literature (2). However, the direct consumption of green beans as food appears in the literature only 1,000 years ago. The custom of picking green pods and selling them in the marketplace was recorded in the Song dynasty during the 12th century. At that time, roasted and boiled fresh green soybeans were used as snacks (2). Ancient Chinese literature mentions the popularity of *maodou* in Jiangsu, Zhejiang, Hunan, and Hubei provinces, indicating that the historical major production areas for *maodou* were probably the lower and middle Changjiang (Yangtze) valley (16).

Today, the majority of *maodou* is produced in the Changjiang river valley including Jiangsu, Shanghai, Zhejiang, Anhui, Jiangxi, Hunan, Hubei, and Sichuan provinces. Citizens in this region consume *maodou* regularly and support considerable fresh markets for the vegetable, especially during summer and fall seasons. Farmers sell *maodou* in the form of shelled seed, unshelled pods, and whole plants with pods attached. The total hectarage of *maodou* in this region is about 100,000 ha. The fresh pod yield is about

4.5–6.0 t/ha for spring planted and 6.0–7.5 t/ha for summer planted *maodou* cultivars. Another *maodou* production area is the southeast coast, including Taiwan, Fujian, and Guangdong provinces. The planting area is more than 30,000 ha and the yield varies from 4.5–9.0 t/ha (17). This area supports almost year-round *maodou* production. Northern and northeastern provinces, such as Shandong, Henan, Tianjing, Beijing, Liaoning, Jilin, and Heilongjiang, produce a small quantity of *maodou* (2).

Maodou breeding has been a focus in Taiwan, especially at the Asian Vegetable Research and Development Center (AVRDC). *Maodou* has not been emphasized at the national level in mainland China. However, about 50 *maodou* cultivars were released by provincial (local) breeding programs in China by 1995 (Tables 14.1 and 14.2). In addition to released cultivars, traditional landraces continue to account for a small portion of the *maodou* market today in southern China. Both public and private companies are involved in *maodou* cultivar development and marketing.

Cultivars for Soy Sauce, Doujiang, Douchi, and Medicine. There are a number of fermented soyfoods in China, including liquid soy sauce, *doujiang* (a thick soy paste), and *douchi* (a fermented and salted whole-bean food). Good-quality soybeans for fermented food processing should have small seeds (100-seed weight less than 15 g), a characteristic aroma and flavor when prepared, and a soft texture. High sugar content is preferred. Cultivars for medicinal use often have a black seedcoat and a green or yellow cotyledon. Several cultivars have been released for medicinal purposes (Table 14.2). For example, Jin Dou 7 Hao and Yu Dou 4 Hao were developed for medicinal use; Xiang B68 was developed for medicinal use and for *douchi*. Jin jiang da Qing Ren was developed for medicinal use and for soy sauce.

Cultivars with Improved Seed Composition. Although high-protein and high-oil cultivars have uses other than traditional soyfoods, high protein can be desirable for tofu and soymilk. Among Chinese soybean cultivars, regional differences in seed composition are large. Northern soybean cultivars are relatively high in seed oil content while southern soybean cultivars are relatively high in protein content (18). Three major cropping systems exist in central and southern China, and are identified by the time of planting (spring, summer, and fall). Cultivars adapted to these contrasting cropping systems differ in seed composition. Spring-planted soybean cultivars have relatively low protein content; fall-planted soybean cultivars have relatively high protein content. Summer-planted soybean cultivars are intermediate in protein content. Most high-protein-content cultivars (i.e., > 45% protein) were developed from southern breeding programs, whereas most high-oil-content cultivars (i.e., > 23% oil) were released from northern breeding programs (Tables 14.1 and 14.2). High seed protein and oil contents have been major breeding objectives since 1986 in China. Landraces with protein content over 52% or oil content over 23% have been identified through large screening programs (19,20). Landraces with extremes in seed protein and oil content are being used in current breeding efforts.

Soybean and Soyfoods in North America

Introduction of Soybean

The soybean was first introduced from China into North America by Samuel Bowen in 1765, to produce soy sauce, and by Benjamin Franklin in 1770, presumably to produce forage and build soil (4). Early in the 20th century, plant explorers Dorsett and Morse returned from China with the first large genetic collection of soybean (more than 4,000 landraces) and founded modern soybean production in the United States (21). This early soybean production in the United States was not for human food but for forage. The discovery of soybean as an important source of oil, about 1915, permanently changed the focus of soybean production in the United States from forage to seed crop. By 1930, 50% of the soybean crop was grown for seed. By 1950, the transition to seed crop was nearly complete. Soybean breeding in the United States was well established by the early 1930s (22) at state agricultural experimental stations and at the U.S. Department of Agriculture (USDA). The primary breeding objectives of these programs were high yield, disease resistance, and broad adaptation.

Current Soyfoods Markets

Most specialty soyfoods cultivars bred and grown in the United States are exported to Japan. However, sizable populations of Asian descent live in many large U.S. cities, and they continue to consume a wide array of soyfoods products purchased at Asian-oriented specialty food stores (23). Tofu and soymilk have made inroads in mainstream supermarkets and now appear in most large stores. Frozen green vegetable soybeans can be purchased in major food stores as well. The vegetarian section of the frozen foods aisle is also a popular place to encounter soyfoods. Soy-based hot dogs, hamburgers, chicken nuggets, and related items are reportedly increasing sales, derived in part from perceived health benefits of soybean consumption. In addition to mainstream supermarkets, health food stores now commonly stock a wide array of soyfoods.

Modern Soyfoods Cultivars

Public breeders have been very active in the development of specialty soyfoods cultivars in North America. The first North American soyfoods cultivar, Kanrich, was released in 1956 for tofu production. Since then, more than 120 public soyfoods cultivars have been released. About two-thirds of all North American soyfoods cultivars were released after 1990, and they account for one-third of all public cultivar releases in that same time period. (Tables 14.3 and 14.4). Most of these cultivars were developed for the Japanese soyfoods export market. The majority of North American soyfoods cultivars were developed in the northern United States and Canada (Table 14.3). Private companies also are involved to a lesser degree, but no data are available on private-sector breeding of soyfoods cultivars.

TABLE 14.3

Distribution of Releases of 123 Public Soyfood Cultivars Developed in North America from 1956 to 2000

Primary specialty trait(s) ^a	Release era					Region		Total
	50s	60s	70s	80s	90s	North	South	
Large seed	1	2	3	4	27	36	1	37
Small seed				11	32	37	6	43
High protein		6		5	6	16	1	17
High protein, large seed		1	1	1	5	8		8
Reduced lipoxygenase					9	9		9
High protein, low lipoxygenase					5	5		5
Low linolenic acid oil					1		1	1
Low palmitic, low linolenic acid oil ^b					1		1	1
Yellow hila, high yield					1	1		1
Null Kunitz trypsin inhibitor				1		1		1
Total	1	9	4	22	87	113	10	123

^aSpecialty trait(s) mentioned in release or registration.

^bSatelite, a cultivar with a low concentration of both palmitic and linolenic acids, was released in 2001.

TABLE 14.4

Origin and Description of 123 Public Soyfood Cultivars Released in North America from 1956 to 2000

Name	MG ^a	Origin	Year ^b	Specialty trait(s)	Reference
AC Colibri	0	Ottawa	1995	Small seed	1997. <i>Can. J. Plant Sci.</i> 77:113–114.
AC Colombe	–2	Ottawa	1996	Small seed	1998. <i>Can. J. Plant Sci.</i> 78:311–312.
AC Hercule	–1	Ottawa	1995	Small seed	1997. <i>Can. J. Plant Sci.</i> 77:257–258.
AC Pinson	–1	Ottawa	1995	Small seed	1996. <i>Can. J. Plant Sci.</i> 76:803–804.
AC Proteina	–1	Ottawa	1997	High protein	1999. <i>Can. J. Plant Sci.</i> 79:109–110.
AC Proteus	–1	Ottawa	1993	High protein	1996. <i>Can. J. Plant Sci.</i> 76:153–154.
Camp	5	Virginia	1989	Small seed	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Canatto	–2	Ottawa	1985	Small seed	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Chico	–1	Minnesota	1983	Small seed	1985. <i>Crop Sci.</i> 25:711.
Danatto	0	North Dakota	1996	Small seed	1997. <i>Crop Sci.</i> 37:1021.
Disoy	1	Iowa	1967	High protein, large seed	1967. <i>Crop Sci.</i> 7:403.
Electron	–1	Ottawa	1999	Small seed	2000. <i>Can. J. Plant Sci.</i> 80:825–826.

(Continued)

TABLE 14.4*(Cont.)*

Name	MG ^a	Origin	Year ^b	Specialty trait(s)	Reference
Emerald	4	Delaware	1975	Large green seed	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Faucon	-1	Ottawa	1999	Small seed	2000. <i>Can. J. Plant Sci.</i> 80:823-824.
Grande	0	Minnesota	1976	Large seed	1977. <i>Crop Sci.</i> 17:824-825.
Harovinton	1	Harrow	1989	Large seed	1991. <i>Can. J. Plant Sci.</i> 71: 525-526.
Heron	-1	Ottawa	1999	Small seed	2000. <i>Can. J. Plant Sci.</i> 80:821-822.
HP201	1	Iowa	1988	High protein	1990. <i>Crop Sci.</i> 30:1361-1362.
HP202	1	Iowa	1988	High protein	1990. <i>Crop Sci.</i> 30:1362.
HP203	1	Iowa	1988	High protein	1990. <i>Crop Sci.</i> 30:1362.
HP204	1	Iowa	1988	High protein	1990. <i>Crop Sci.</i> 30:1363.
IA1002	1	Iowa	1991	High protein, low lipoxxygenase	See Iowa State Univ. web site (25).
IA1003	1	Iowa	1991	High protein, low lipoxxygenase	See Iowa State Univ. web site (25).
IA1005	1	Iowa	1994	Large seed	See Iowa State Univ. web site (25).
IA1007	1	Iowa	1997	Large seed	See Iowa State Univ. web site (25).
IA2005	2	Iowa	1991	Small seed	See Iowa State Univ. web site (25).
IA2009	2	Iowa	1991	High protein, low lipoxxygenase	See Iowa State Univ. web site (25).
IA2010	2	Iowa	1991	High protein, low lipoxxygenase	See Iowa State Univ. web site (25).
IA2011	2	Iowa	1993	High protein, low lipoxxygenase	See Iowa State Univ. web site (25).
IA2012	2	Iowa	1993	Large seed	See Iowa State Univ. web site (25).
IA2013	2	Iowa	1993	Large seed	See Iowa State Univ. web site (25).
IA2016	2	Iowa	1994	Large seed	See Iowa State Univ. web site (25).
IA2017	2	Iowa	1994	Large seed	See Iowa State Univ. web site (25).
IA2018	2	Iowa	1994	Large seed	See Iowa State Univ. web site (25).
IA2019	2	Iowa	1994	Large seed	See Iowa State Univ. web site (25).
IA2020	2	Iowa	1994	Large seed	See Iowa State Univ. web site (25).
IA2023	2	Iowa	1995	Small seed	See Iowa State Univ. web site (25).
IA2024	2	Iowa	1995	Small seed	See Iowa State Univ. web site (25).
IA2025	2	Iowa	1996	Triple-null lipoxxygenase	See Iowa State Univ. web site (25).
IA2027	2	Iowa	1996	Triple-null lipoxxygenase	See Iowa State Univ. web site (25).
IA2028	2	Iowa	1996	Triple-null lipoxxygenase	See Iowa State Univ. web site (25).
IA2029	2	Iowa	1996	Triple-null lipoxxygenase	See Iowa State Univ. web site (25).

Name	MG ^a	Origin	Year ^b	Specialty trait(s)	Reference
IA2030	2	Iowa	1996	Triple-null lipoxygenase	See Iowa State Univ. web site (25).
IA2032	2	Iowa	1996	Triple-null lipoxygenase	See Iowa State Univ. web site (25).
IA2033	2	Iowa	1996	Triple-null lipoxygenase	See Iowa State Univ. web site (25).
IA2034	2	Iowa	1996	Large seed	See Iowa State Univ. web site (25).
IA2035	2	Iowa	1997	Small seed	See Iowa State Univ. web site (25).
IA2036LF	2	Iowa	2000	Lipoxygenase free	See Iowa State Univ. web site (25).
IA2037	2	Iowa	1997	Large seed	See Iowa State Univ. web site (25).
IA2040	2	Iowa	1998	Large seed	See Iowa State Univ. web site (25).
IA2041	2	Iowa	1998	Large seed	See Iowa State Univ. web site (25).
IA2042	2	Iowa	1998	Large seed	See Iowa State Univ. web site (25).
IA2043	2	Iowa	1999	Large seed	See Iowa State Univ. web site (25).
IA2044	2	Iowa	1999	Large seed, high protein	See Iowa State Univ. web site (25).
IA2045	2	Iowa	1999	Large seed	See Iowa State Univ. web site (25).
IA2046	2	Iowa	1999	Large seed, high protein	See Iowa State Univ. web site (25).
IA2047	2	Iowa	1999	Large seed, high protein	See Iowa State Univ. web site (25).
IA2048	2	Iowa	1999	Large seed, high protein	See Iowa State Univ. web site (25).
IA2049	2	Iowa	1999	Large seed, high protein	See Iowa State Univ. web site (25).
IA2053	2	Iowa	2000	Large seed	See Iowa State Univ. web site (25).
IA2054	2	Iowa	2000	Large seed	See Iowa State Univ. web site (25).
IA2055	2	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA2056	2	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA2057	2	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA2058	2	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA2059	2	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA2060	2	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA2061	2	Iowa	2000	Yellow hila, high yield	See Iowa State Univ. web site (25).
IA3001	3	Iowa	1993	High protein	See Iowa State Univ. web site (25).
IA3002	3	Iowa	1993	Large seed	See Iowa State Univ. web site (25).
IA3006	3	Iowa	1995	Large seed	See Iowa State Univ. web site (25).
IA3007	3	Iowa	1995	Small seed	See Iowa State Univ. web site (25).
IA3008	3	Iowa	1997	Small seed	See Iowa State Univ. web site (25).
IA3009	3	Iowa	1997	Large seed	See Iowa State Univ. web site (25).
IA3011	3	Iowa	1998	Large seed	See Iowa State Univ. web site (25).
IA3012LF	3	Iowa	2000	Triple-null lipoxygenase	See Iowa State Univ. web site (25).
IA3013	3	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IA4001	4	Iowa	1995	Small seed	See Iowa State Univ. web site (25).
IA4002	4	Iowa	2000	Small seed	See Iowa State Univ. web site (25).
IL1	2	Illinois(Urbana)	1989	Small seed	1991. <i>Crop Sci.</i> 31:233–234.
IL2	3	Illinois(Urbana)	1989	Small seed	1991. <i>Crop Sci.</i> 31:234.

(Continued)

TABLE 14.4

(Cont.)

Name	MG ^a	Origin	Year ^b	Specialty trait(s)	Reference
Kahala	4	Hawaii	1969	High protein	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Kaikoo	4	Hawaii	1969	High protein	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Kailua	4	Hawaii	1969	High protein	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Kanrich	3	Iowa	1956	Large seed	1966. <i>Crop Sci.</i> 6:391.
Kunitz	3	Illinois(Urbana)	1989	Kunitz trypsin inhibitor null	1991. <i>Crop Sci.</i> 31:232–233.
LN92-7369	2	Illinois(Urbana)	1999	High protein	2000. <i>Crop Sci.</i> 40:296.
LS201	2	Iowa	1989	Large seed	1990. <i>Crop Sci.</i> 30:1363.
LS301	3	Iowa	1987	Large seed	1990. <i>Crop Sci.</i> 30:1363–1364.
Magna	2	Iowa	1967	High protein	1967. <i>Crop Sci.</i> 7:403.
Marion	2	Iowa	1976	Large seed	1977. <i>Crop Sci.</i> 17:824.
Mercury	2	Nebraska	1994	Small seed	1995. <i>Crop Sci.</i> 35:1205.
Merrimax	0	New Hampshire	1986	Large seed, vegetable	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Micron	–1	Ottawa	1995	Small seed	1997. <i>Can. J. Plant Sci.</i> 77:115–116.
Minnatto	0	Minnesota	1989	Small seed	1991. <i>Crop Sci.</i> 31:233.
Mokapu Summer	4	Hawaii	1969	High protein	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
N6201	6	North Carolina	2000	Large seed	2003. <i>Crop Sci.</i> 43:1125–1126.
N7101	7	North Carolina	2000	Small seed	2003. <i>Crop Sci.</i> 43:1127–1128.
N7102	7	North Carolina	2000	Small seed	2003. <i>Crop Sci.</i> 43:1128–1129.
N7103	7	North Carolina	2000	Small seed	2003. <i>Crop Sci.</i> 43:1128.
Nattawa	0	Ottawa	1981	Small seed	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Nattosan	0	Ottawa	1989	Small seed	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Norpro	0	North Dakota	1998	Large seed, tofu type	1999. <i>Crop Sci.</i> 39:591.
Ohio FG1	3	Ohio	1994	Large seed, tofu type	1996. <i>Crop Sci.</i> 36:813.
Ohio FG2	3	Ohio	1994	Large seed, tofu type	1996. <i>Crop Sci.</i> 36:814.
Pearl	6	North Carolina	1994	Small seed	1995. <i>Crop Sci.</i> 35:1713.
Prize	2	Iowa	1967	Large seed	1967. <i>Crop Sci.</i> 7:404.
Prolina	6	North Carolina	1996	High protein	1999. <i>Crop Sci.</i> 39:294–295.
Protana	2	Indiana	1969	High protein	1971. <i>Crop Sci.</i> 11:312.
Proto	0	Minnesota	1989	High protein	1991. <i>Crop Sci.</i> 31:486.
Satelite	6	North Carolina	2001	Low palmitic, low linolenic	Notice of Release.
Saturn	3	Nebraska	1994	Large seed, edamame, tofu	1995. <i>Crop Sci.</i> 35:1205.
Soyola	6	North Carolina	2000	Low linolenic acid	USDA GRIN (24) and Notice of Release.

Name	MG ^a	Origin	Year ^b	Specialty trait(s)	Reference
SS201	2	Iowa	1989	Small seed	1990. <i>Crop Sci.</i> 30:1361.
SS202	2	Iowa	1989	Small seed	1990. <i>Crop Sci.</i> 30:1361.
T2653	-1	Ottawa	1995	Small seed	1996. <i>Can. J. Plant Sci.</i> 76:805-806.
TNS	-1	Ottawa	1995	Small seed	1997. <i>Can. J. Plant Sci.</i> 77:117-118.
Toyopro	0	Minnesota	1995	High protein	1997. <i>Crop Sci.</i> 37:1386.
UM-3	0	Minnesota	2000	Small seed	2000. <i>Crop Sci.</i> 40:1826-1827.
Vance	5	Virginia	1986	Small seed	USDA GRIN (24) and Bernard <i>et al.</i> , 1988 (22).
Verde	3	Delaware	1967	Large seed	1971. <i>Crop Sci.</i> 11:312.
Vinton	1	Iowa	1977	High protein, large seed	1980. <i>Crop Sci.</i> 20:673-674.
Vinton 81	1	Iowa	1981	Large seed, high protein	1984. <i>Crop Sci.</i> 24:384.

^aU.S. maturity group designation. For ease of calculation and representation, maturity group data are presented in Arabic rather than standard Roman numerals, where 000 = -2, 00 = -1, 0 = 0, I = 1, II = 2, III = 3, and so on. Decimal values do not refer to the maturity classification system known as relative maturity groupings employed by U.S. breeders. Rather, they reflect a simple average of traditional maturity group ratings. For example, the mean maturity of five cultivars of maturity group I and five cultivars of maturity group II is 1.5.

^bYear of release.

Genetic Base and Diversity of Soyfoods Cultivars

Introduction of soyfoods traits into breeding has often been achieved through the mating of exotic germplasm with adapted breeding stock. This strategy has produced a genetic base for soyfoods cultivars that is substantially different from that of commodity cultivars. Soyfoods cultivars receive about a quarter of their pedigree from ancestors that were virtually absent in the genetic base of commodity cultivars. In total, 29 unique "soyfoods ancestors" were used in the development of North American soyfoods cultivars (Table 14.5). At least eight new ancestors (PI 153293, PI 159925, PI 189880, PI 257435, PI 261475, PI 90406, PI 92567, and T215) had high seed-protein content (> 44% on a dry weight basis). Thirteen soyfoods ancestors (H-24, JA42, Jizuka, PI 189950, PI 196176, PI 408016B, PI 437267, PI 437296, the unknown small-seeded parents of Vance and Danatto, PI 101404, PI 135624, and PI 81762) were used for small-seeded cultivar development. The latter three are accessions of wild soybean (*G. soja*), the small-seeded progenitor of cultivated soybean. The Japanese cultivars Aoda, Jogun, and Nakasennari have been important exotic sources of large seed size. It is interesting to note that no single soyfoods ancestor has dominated soyfoods cultivar breeding. The broad genetic base for soyfoods cultivars reflects the wide range of breeding objectives applicable to soyfoods and the wide range of maturity groups for which they are bred.

At present, soyfoods cultivars that diverge most from commodity cultivars (in terms of pedigree) tend to be low-yielding (most yield less than 90% of commodity types), and for this reason have rarely been used as parents in breeding for commodity cultivars. However, continuing selection for improved yield has produced recent soyfoods types that yield only slightly lower than commodity cultivars. For example,

TABLE 14.5

Ancestors of North American Soybean That Contribute to Soyfood Cultivars but Do Not Contribute Significantly to Commodity Cultivars^a

Ancestor	Specialty trait(s)	Genetic contribution to soyfoods base, %	Genetic contribution to commodity base ^b , %
Kanro	Large seed	3.645	0.025
Jogun	Large seed	3.614	0.024
Unknown male parent of Vance	Small seed	2.062	0.000
Bansei	High protein content	2.062	0.001
PI 101404, <i>G. soja</i>	Small seed	1.740	0.000
H-24	Small seed	1.546	0.000
Jizuka	Small seed	1.031	0.000
PI 196176	Small seed	1.031	0.000
Aoda	Large seed	1.031	0.000
PI 437267	Small seed	0.773	0.000
PI 86023	Null lipoxxygenase-lx ₂	0.644	0.000
Nakasennari	Large seed	0.515	0.000
PI 81762, <i>G. soja</i>	Small seed	0.515	0.000
PI 408016B	Small seed	0.515	0.000
Unknown male parent of Danatto	Small seed?	0.515	0.000
PI 135624, <i>G. soja</i>	Small seed	0.451	0.000
PI 261475	High protein content	0.451	0.000
PI 189880	High protein content	0.387	0.000
DSR 252	High yield?	0.322	0.000
Pridesoy II	High yield?	0.258	0.000
PI 153293	High protein content	0.258	0.000
T215	High protein content	0.258	0.000
PI 437296	Small seed	0.258	0.000
PI 65338 ^c	Low protein content	0.258	0.001
Hahto	Green seed coat, high protein content	0.258	0.001
JA42	Small seed	0.161	0.000
PI 123440	Low linolenic acid content	0.129	0.000
PI 189950	Small seed	0.129	0.000
PI 92567	High protein and oleic acid content	0.032	0.000
PI 90406	High protein and oleic acid content	0.032	0.000
PI 157440	Null Kunitz inhibitor	0.016	0.000
Total genetic contribution		24.897	0.052

^aThe approximate genetic contribution of 31 "soyfood ancestors" to 89 North American soyfood cultivars released from 1956 to 2000 was estimated from pedigree analysis.

^bKanro, Jogun, Bansei, Hahto, and PI 65338 contributed predominantly to soyfood cultivars rather than to commodity cultivars. The other 29 ancestors contributed exclusively to soyfood cultivars.

^cPI 65338 itself is a low protein content accession. It appeared in the pedigree of a high protein content cultivar, Protana.

small-seeded cultivar N7103 and large-seeded cultivar N6201 yield only about 5 and 8% below commodity cultivars, respectively. Thus, soyfoods cultivars may become a more important source of diversity for commodity breeding in the future.

The potential utility of soyfoods cultivars in commodity breeding is supported by comparing genetic diversity of soyfoods and commodity soybean cultivars, using coefficient of parentage (CP) analysis. Coefficient of parentage is a form of numerical taxonomy (or pedigree tracking) that uses familial relationships among cultivars to calculate the approximate proportion of genes that cultivars share in common (26). A CP value of 0 indicates no pedigree relationship between two cultivars (i.e., no ancestors in common), whereas values of 0.25, 0.50, and 1.0 indicate half sib, full sib, and identical twin relationships, respectively. Summarizing results for North American cultivars, CP analysis shows that (a) soyfoods cultivars are at least as diverse, as a group, as are commodity cultivars, both in the Midwest and South, and (b) soyfoods cultivars are not closely related, as a group, to commodity cultivars. These results indeed suggest that soyfoods cultivars are a potential reservoir of genetic diversity for commodity breeding. For those breeders familiar with CP analysis, the authors elaborate here by noting that average CP relations *within* soyfoods and commodity groups are 0.15 and 0.18 in the Midwest, and 0.18 and 0.24 in the South, respectively. Average CP relations between these two groups are 0.12 in the North and 0.17 in the South.

For breeders who are more interested in soyfoods cultivar development than commodity cultivar development, CP analysis continues to be useful in that it helps identify desirable parental combinations. Desirable is defined here as diverse, but possessing somewhat similar soyfoods characteristics. To illustrate the utility of CP analysis, pedigree relations among North American specialty cultivars were depicted graphically (Fig. 14.1). Distance on the graph indicates diversity or genetic distance between cultivars, based on multidimensional scaling analysis. The distinction between northern and southern specialty-use cultivars is clearly seen, with the nine southern soyfoods cultivars appearing in the lower-right quadrant of the graph and other cultivars scattered broadly over the rest of the graph area. Other patterns are apparent, and one can superimpose cluster analysis over CP analysis to describe them. Cluster analysis subdivides cultivars into groups or 'clusters' of closely related genotypes. The authors found that U.S. soyfoods cultivars could be separated into seven readily identifiable clusters (Fig. 14.1). These clusters have meaning in terms of choosing parents for mating, which can be illustrated in the following cluster descriptions: Cluster 1 is small-seeded cultivars of maturity group I or II. Clusters 2 and 3 are small-seeded cultivars of maturity group II or III. Cluster 4 is large-seeded cultivars of maturity group II or III. Cluster 5 is large-seeded, high protein content, and null Kunitz inhibitor cultivars of maturity group III or IV. Cluster 6 is small-seeded and high protein content cultivars of maturity group III or IV. Cluster 7 is southern cultivars of maturity group IV or later.

Although the statistical approach above may be a bit difficult to follow, the implications are not. Soyfoods breeders have a great opportunity to take advantage of diversity patterns shown here and avoid the mating of parents that are too closely related and, thus, unlikely to produce exceptional cultivars. That is, breeders can avoid

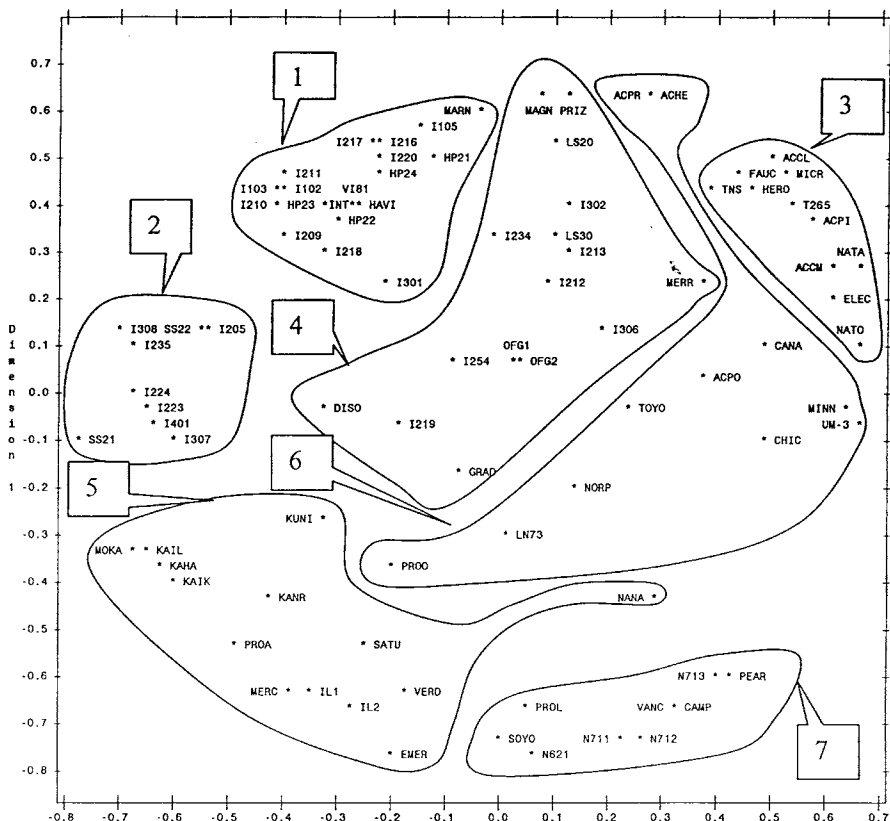


Figure 14.1. Two-dimensional representation of genetic relationships among 89 soyfood cultivars derived from a two-dimensional multidimensional scaling (MDS) analysis based on coefficient of parentage (CP). The stress value for the two-dimensional MDS analysis was 0.35 and the regression R^2 of fitted similarity on the original CP was 0.43. The CP between any two cultivars can be estimated as $(1 - \text{linear distance between them})$, where 1 is the maximum CP relation between clusters. Distances ≥ 1 indicate no relationship. Clusters were superimposed upon the graph to clarify geographical interpretation of the analysis and designated as Clusters 1 through 7.

mating progeny that belong to the same cluster and focus instead on cross-breeding between clusters. For example, intermating among Cluster 1, 2, or 3 should be a wise choice for small-seed breeding efforts in the Midwest. For high-protein breeding, mating between high-protein cultivars from Clusters 5 and 6 might be a good choice.

For the sake of clarity, the authors mention here that clusters were identified using Ward's minimum variance method. Comparison of clustering precision here with that from previous studies confirmed that the clusters were well defined, statistically, and were therefore useful descriptors of diversity (18,27). For those familiar with CP analysis, average CP within clusters were larger than 0.25 for Clusters 1, 2, 3, and 7 and smaller than 0.25 between all clusters.

Soybean and Soyfoods in Japan

Introduction of Soybean to Japan

The history of soybean cultivation in Japan can be traced back to the early Yayoi culture around 0 AD (28,29). It is believed that soybean was introduced to Japan from China or Korea via human migration (30). Because wild soybean, *Glycine soja*, is widely distributed in Japan and rich in genetic diversity, it is believed that hybridization of the Chinese or Korean introductions with native wild soybean populations may have played a major role in the development of various Japanese soybean landraces over a long period of time. Molecular analysis of modern Chinese, Japanese, and North American cultivars indicated that Japanese cultivars are quite distinct from cultivars of other regions (6,31).

Traditional Soyfoods in Japan

The soybean has long been important in the Japanese diet. Although there are many traditional soyfoods, they can be classified into three groups, based on the stage of development of the soybean when it is consumed: immature, mature, and sprouting. Immature soybean is consumed as a vegetable soybean (*edamame*), which is typically harvested and sold as green pods attached to the stem. Although *edamame* is a nutritious vegetable, it is also highly appreciated by many beer drinkers, especially in the summer season, when *edamame* is consumed with beer in much the same way that salted peanuts are consumed in the United States. Soybean sprouts (*moyashi*) are consumed raw as a vegetable in salads or cooked in Chinese-style dishes.

The mature soybean is used in various traditional foods in Japan: soymilk (*tonyu*), soybean curd (tofu), frozen soybean curd (*kori-dofu*), thin fried soybean curd (*aburage*), thick fried soybean curd (*ganmodoki*), baked soybean curd (*yaki-dofu*), and *yuba*, which is a very tasty soyfoods product made by skimming and drying the thick creamy layer that forms on the surface of heated soymilk. Large-seeded soybeans with a yellow seedcoat can be used to produce a boiled soybean dish (*nimame*), and large-seeded soybeans with a black seedcoat are boiled as a traditional New Year's food (*kuromame*). Small-seeded soybeans are used for fermented soybean (natto). Soybeans with medium-sized seeds are used for the production of soybean paste (*miso*), after boiling and fermentation. Roasted soybean (*iri-mame*) is important for traditional ceremonies at the beginning of spring. Yellow or green soybean meal (*kinako*) is used for confectionery. Defatted soybean meal can be fermented to produce soy sauce (*shoyu*).

Current Soyfoods Markets

Though soybean is used for various purposes in Japan, vegetable oil production accounts for almost 80% of the total soybean consumption. During the past decade (1991 to 2000), the total annual soybean consumption in Japan was estimated at around 4.8 million tons, out of which about 3.7 million tons were used for vegetable oil production. Since annual domestic soybean production during this period was only 160,000 tons, soybean imported from the United States, Brazil, Canada, China, and other nations accounted for more than 95% of the total consumption. For the traditional soyfoods, annual estimated consumption

is as follows: about 500,000 tons for tofu and related products, 160,000 tons for *miso*, 120,000 tons for natto, 30,000 tons each for soy sauce and frozen tofu, and 20,000 tons for *nimame* (32). Soybean produced specifically for soyfoods in Japan is mainly used to make high-quality *nimame*, *miso*, and tofu. At present, no transgenic (also known as genetically modified organism or GMO) soybean is accepted in the soyfoods market.

Modern Soyfoods Cultivars

Modern soybean breeding began at agricultural experiment stations in Japan by selecting true-breeding cultivars from segregating landraces in the 1910s. Crossbreeding was introduced about 1916 (33). In 1935, Akita, Ibaraki, and Kumamoto Prefectures initiated soybean breeding, with funding from the national government. The soybean breeding system in Japan has been reorganized several times since then and there are now seven soybean breeding laboratories: two in Hokkaido, and one each in Tohoku, Tsukuba, Nagano, Chugoku, and Kyushu. Soybean breeding has been carried out mainly by the public sector in Japan, except for development of *edamame* cultivars, which has been actively pursued by the private sector.

Although the main objective of soybean breeding prior to World War II was improved oil production, objectives have changed in recent decades with the increasing reliance upon imported soybean (33). Today, the major objectives are high seed-protein content and good soyfoods, as described in the following sections (34).

TABLE 14.6

Distribution of Release of 97 Specialty-Use Public Soyfoods Cultivars Developed in Japan from 1950 to 1995^a

Primary specialty use or trait	Release era					Region ^b			Total
	0s	60s	70s	80s	90s ^c	NJ	CJ	SJ	
Large seed		5	7	9	5	15	11		26
Small seed				3	1	2	2		4
Tofu	13	33	14	18	8	25	45	16	86
Natto				3	1	2	2		4
Miso	9	16	5	4	3	11	26		37
Nimame		5	7	9	5	15	11		26
Confection				1	1	2			2
Soymilk with low lipooxygenase					1		1		1
Fodder, green manure		2	1				2	1	3
Total	22	61	34	47	25	72	100	17	189

^aData are for the three major three growing regions of Japan: northern Japan (NJ), central Japan (CJ), and southern Japan (SJ). Totals add to more than 97 because many cultivars were released for more than one specialty trait or purpose.

^bCentral Japan includes Honshu island from Chugoku to Tohoku (~35–41°N), Northern Japan includes Hokkaido island (~42–45° N), and Southern Japan is Kyushu Island (~31–34° N)

^c90s refers to 1990 through 1995.

TABLE 14.7

Origin and Description of 97 Public Soyfood Cultivars Developed and Released in Japan from 1950 to 1995

Name	Approximate U.S.maturity group	Japanese maturity group ^a	Developing institution ^b	Year of release	Specialty use
Aki Sengoku	IX	Vc	Kumamoto (Aso)	1962	Tofu
Akishirome	V	IIIc	Kyushu (Kumamoto)	1979	Tofu
Akiyosh	VIII	IVc	Kumamoto (Aso)	1963	Tofu
Aso Aogari	VII	Vc	Kumamoto (Aso)	1963	Fodder, green manure
Aso Masari	IX	Vc	Kumamoto (Aso)	1954	Tofu
Aso Musume	VIII	Vc	Kumamoto (Aso)	1956	Tofu
Ayahikari	I	IIc	Nagano (Chuchin)	1991	Tofu, <i>miso</i> , <i>nimame</i>
Bon Minori	I	IIa	Ibaraki (Ishioka)	1961	Tofu, <i>miso</i>
Daruma Masari	I	IIc	Akita (Odate)	1951	<i>Miso</i> , tofu
Dewa Musume	II	IIc	Tohoku (Kariwano)	1977	Tofu
Enrei	III	IIc	Nagano (Kikyogahara)	1971	Tofu, <i>miso</i> , <i>nimame</i>
Fuji Musume	I	IIa	Saga	1961	Tofu
Fuji Otome	IV	IIb	Ibaraki (Ishioka)	1966	Tofu, <i>miso</i>
Fujimijiro	III	IIc	Nagano (Kikyogahara)	1964	Tofu, <i>miso</i>
Fuku Shirome	II	IIb	Tohoku (Kariwano)	1985	Tofu
Fukumejiro	I	IIb	Ibaraki (Ishioka)	1958	Tofu, <i>miso</i>
Fukunagaha	II	IIa	Hokkaido (Central)	1981	<i>Nimame</i> , tofu
Fukuyutaka	VII	IVc	Kyushu (Kumamoto)	1980	Tofu
Ginrei	V	IIIc	Nagano (Chushin)	1995	<i>Miso</i>
Gogaku	VIII	IVc	Kumamoto (Aso)	1967	Tofu
Hatsukari	II	IIb	Tohoku (Kariwano)	1959	<i>Miso</i> , tofu
Higo Musume	00	IIa	Saga	1965	Tofu
Himeshirazu	VII	Vc	Nat.Inst.Animal Industry	1970	Fodder, green manure
Himeyutaka	I	Ib	Hokkaido (Tokachi)	1976	<i>Nimame</i> , tofu
Hokkai Hadaka	00	Ia	Hokkaido (Tokachi)	1958	Tofu, <i>miso</i>
Hougyoku	IX	Vc	Kumamoto (Aso)	1953	Tofu
Hourai	0	Ib	Hokkaido (Tokachi)	1965	Tofu, <i>miso</i>
Hourei	II	IIb	Nagano (Chushin)	1987	Tofu
Hyuuga	VIII	IVc	Kumamoto (Aso)	1969	Tofu
Karikachi	I	Ia	Hokkaido (Tokachi)	1959	Tofu, <i>miso</i>
Kariyutaka	I	Ib	Hokkaido (Tokachi)	1991	<i>Nimame</i> , tofu, <i>miso</i>
Karumai	III	IIb	Tohoku (Kariwano)	1973	Tofu
Kitahomare	II	Ib	Hokkaido (Tokachi)	1980	Tofu, <i>miso</i>
Kitakomachi	00	Ia	Hokkaido (Tokachi)	1978	<i>Nimame</i> , tofu
Kitamusume	I	Ib	Hokkaido (Tokachi)	1968	Tofu, <i>miso</i>
Kogane Daizu	0	IIa	Saga	1958	Tofu
Kogane Jiro	0	Ib	Hokkaido (Tokachi)	1961	Tofu, <i>miso</i>
Kokeshi Jiro	II	IIb	Ibaraki (Ishioka)	1964	Tofu, <i>miso</i>

(Continued)

TABLE 14.7 (cont'd)
(Cont.)

Name	Approximate U.S.maturity group	Japanese maturity group ^a	Developing institution ^b	Year of release	Specialty use
Komamusume	I	Ib	Hokkaido (Central)	1982	<i>Nimame</i> , tofu
Kosuzu	III	IIc	Tohoku (Kariwano)	1987	<i>Natto</i>
Misuzu Daizu	V	IIc	Nagano (Kikyogahara)	1968	Tofu, <i>miso</i> , <i>nimame</i>
Miyagi Oojiro	VI	IIc	Nagano (Kikyogahara)	1978	<i>Nimame</i>
Mutsu Mejiro	I	IIb	Tohoku (Kariwano)	1965	Tofu
Mutsu Shiratama	II	IIc	Tohoku (Kariwano)	1967	Tofu, <i>nimame</i>
Nagaha Jiro	II	Ib	Hokkaido	1961	Tofu
Nakasennari	V	IIc	Nagano (Kikyogahara)	1978	Tofu, <i>miso</i>
Nanbu Shirome	II	IIc	Tohoku (Kariwano)	1977	Tofu
Nasu Shirome	III	IIc	Nagano (Kikyogahara)	1968	Tofu, <i>miso</i>
Nema Shirazu	III	IIb	Tohoku (Kariwano)	1961	Tofu, <i>nimame</i>
Nishimusume	V	IIc	Kyushu (Kumamoto)	1990	Tofu
Oku Mejiro	IV	Ila	Ibaraki (Ishioka)	1961	Tofu, <i>miso</i>
Oku Shirome	II	IIc	Tohoku (Kariwano)	1972	Tofu
Oosodenomai	I	Ib	Hokkaido (Tokachi)	1992	<i>Nimame</i> , tofu, confection
Ootsuru	IV	IIc	Nagano (Chushin)	1988	Tofu, <i>miso</i> , <i>nimame</i>
Orihime	0	Ila	Saga	1967	Tofu
Oshima Shirome	III	Ila	Hokkaido	1964	Tofu, <i>miso</i>
Raiden	II	IIb	Tohoku (Kariwano)	1966	Tofu
Raikou	II	IIc	Tohoku (Kariwano)	1969	Tofu
Ryuuho	II	IIb	Tohoku (Kariwano)	1995	Tofu
Sayohime	0	Ila	Saga	1960	Tofu
Shin Mejiro	II	IIb	Ibaraki (Ishioka)	1954	Tofu, <i>miso</i>
Shinsei	0	Ia	Hokkaido (Tokachi)	1961	Tofu, <i>miso</i>
Shiro Shennari	II	IIb	Nagano (Kikyogahara)	1971	Tofu, <i>miso</i>
Shiromeyutaka	V	IIc	Nagano (Kikyogahara)	1962	Tofu, <i>miso</i>
Shirotae	VI	IIc	Nagano (Kikyogahara)	1965	Tofu, <i>nimame</i>
Suzuhime	I	Ia	Hokkaido (Tokachi)	1980	<i>Natto</i>
Suzukari	II	IIc	Tohoku (Kariwano)	1985	Tofu, <i>nimame</i>
Suzumaru	0	Ib	Hokkaido (Central)	1988	<i>Natto</i>
Suzunone	II	IIb	Tohoku (Kariwano)	1995	<i>Natto</i>
Suzuyutaka	III	IIc	Tohoku (Kariwano)	1982	Tofu
Tachi Suzunari	II	IIb	Ibaraki (Ishioka)	1960	Tofu, <i>miso</i>
Tachikogane	II	IIb	Tohoku (Kariwano)	1983	Tofu
Tachinagaha	IV	IIc	Nagano (Chusin)	1986	Tofu, <i>miso</i> , <i>nimame</i>
Tachiyutaka	IV	IIc	Tohoku (Kariwano)	1987	Tofu
Tamahikari	V	IIc	Nagano (Kikyogahara)	1971	Tofu, <i>miso</i> , <i>nimame</i>
Tamahomare	VI	IIc	Nagano (Kikyogahara)	1980	Tofu, <i>miso</i>
Tamamusume	II	Ila	Ibaraki (Ishioka)	1950	Tofu, <i>miso</i>
Tanrei	III	IIb	Nagano (Kikyogahara)	1978	Tofu, <i>miso</i>

Name	Approximate U.S.maturity group	Japanese maturity group ^a	Developing institution ^b	Year of release	Specialty use
Tokachi Kuro	I	Ib	Hokkaido (Tokachi)	1984	<i>Nimame</i> , confection
Tokachi Shiro	I	Ib	Hokkaido (Tokachi)	1961	Tofu, <i>miso</i>
Tomoyutaka	II	IIb	Tohoku (Kariwano)	1990	Tofu
Toyohomare	I	Ib	Hokkaido (Tokachi)	1994	<i>Nimame</i> , tofu
Toyokomachi	0	Ia	Hokkaido (Tokachi)	1988	<i>Nimame</i> , tofu
Toyomusume	I	Ib	Hokkaido (Tokachi)	1985	<i>Nimame</i> , tofu
Toyoshirome	VII	IVc	Kyushu (Kumamoto)	1985	Tofu
Toyosuzu	I	Ib	Hokkaido (Tokachi)	1966	<i>Nimame</i> , tofu
Tsurukogane	I	Ib	Hokkaido (Central)	1984	<i>Nimame</i> , tofu
Tsurumusume	I	Ib	Hokkaido (Central)	1990	<i>Nimame</i> , tofu
Tsurusengoku	VIII	Vc	Nat. Inst. Animal Industry	1965	Fodder, green manure
Ugo Daizu	II	IIc	Akita (Odate)	1952	<i>Miso</i> , tofu
Wase Kogane	0	Ib	Hokkaido (Tokachi)	1964	Tofu, <i>miso</i>
Wase Shiroke	0	IIb	Tohoku (Kariwano)	1956	<i>Miso</i> , tofu
Wase Shirome	0	IIb	Tohoku (Kariwano)	1967	Tofu
Wasesuzunari	I	IIb	Tohoku (Kariwano)	1983	Tofu
Yumeyutaka	II	IIc	Ibaraki (Tsukuba)	1992	Soymilk with low lipoxy- genase
Yuuhome	I	Ib	Hokkaido (Central)	1979	<i>Nimame</i> , tofu
Yuuzuru	I	Ib	Hokkaido (Central)	1971	<i>Nimame</i> , tofu

^aJapanese maturity group are denoted by Roman numerals, which represent days from planting to flowering, followed by Arabic characters, which represent days from flowering to maturity.

^bHokkaido, Hokkaido (Central), and Hokkaido (Tokachi) denote locations in Northern Japan; Kyushu (Kumamoto), Kumamoto (Aso), and Saga denote locations in Southern Japan; the others denote locations in Central Japan (35).

Eighty-six publicly released Japanese soyfoods cultivars were developed during the period 1950 to 1988 and a total of 97 by 1995. Japanese soyfoods cultivars are described in [Tables 14.6](#) and [14.7](#) (36). These were developed from 74 ancestors, most of which were traditional soyfoods landraces (37).

Cultivars for Tofu (Soybean Curd) and Soymilk. Soybean cultivars that are most prized for their tofu processing quality usually have an intermediate level for most compositional traits, as exemplified by the Japanese cultivar Fukuyutaka which has about 45% protein and 20% oil (38). In addition to Fukuyutaka, Enrei, and Suzuyutaka are also very desirable for tofu production. Soybean cultivars lacking lipoxxygenase isozymes have recently been developed; these can produce soymilk free from the grassy beany flavor and taste (39,40). Soyfoods products developed from lipoxxygenase-free soybean have been readily accepted by Japanese consumers.

Cultivars for Miso (Soybean Paste). The suitable characteristics of soybean for the production of *miso* are as follows: white hilum color, high water-absorbing capacity under soaking, soft structure, and bright or light yellow color of cooked

beans (38). The composition of free sugars also affects the taste of *miso*. High sugar content, especially sucrose, is preferable, and is positively associated with the good taste of boiled soybeans. While most of the Japanese soybean cultivars can be readily used for producing *miso* (38), Tamahomare is considered to be an especially desirable cultivar.

Cultivars for Natto (Fermented Soybean). For natto processing, soybean with a bright seed surface color, a high water-absorbing capacity, low sucrose content, and high stachyose content is most suitable (38). Small-sized seeds are generally used for high-quality natto, although medium-sized seeds may also produce natto with good taste. Among the Japanese cultivars registered by the Ministry of Agriculture, Forestry and Fisheries (MAFF), Suzumaru and Kosuzu are recognized for production of high-quality natto (35) (Table 14.7). An older cultivar used to make high-quality natto, Natto-shoryu (or Natto-Kotsubu), was selected from a small-seeded landrace in Ibaraki Prefecture by the Ibaraki Agricultural Experiment Station. Natto-shoryu is famous for its small seed size (less than 10 g per 100 seeds). Exact requirements for a natto cultivar tend to vary among manufacturers, reflecting the stratified and complex nature of the natto market.

Cultivars for Nimame (Boiled Soybean). Cultivars with large seeds (more than 30 g per 100 seeds), a yellow seedcoat and hilum, and a total free sugar content above 11% are suitable for *nimame* (41). Among the cultivars registered by the MAFF, Tachinagaha, Toyomusume, and Ootsuru are used for the production of high-quality *nimame* (35) (Table 14.7). In addition, some local cultivars with large-sized seeds such as Miyagi-shirome are also suitable for high-quality *nimame* production. Black-seeded soybeans are used to prepare one of the Japanese New Year's specialty foods. Tanba-guro, which is a local cultivar with round black seeds (more than 60 g per 100 seeds), is produced in Hyogo Prefecture and neighboring areas. Shinano-kuro and Wase-guro, released from Nagano Chushin Agricultural Experiment Station, are also used for black soybean cultivation in the central part of Japan.

Cultivars with Low Allergenic Properties. Low-allergenic soybean cultivars are being developed using two genetic sources: (a) a spontaneous mutant of wild soybean showing a lack, or an extremely low level, of α , α' , and β -subunit bands that compose 7S globulin (42); and (b) a similar mutant induced by gamma-ray irradiation (43,44). These cultivars are expected to be used for the manufacture of hypo-allergenic soybean products as well as various novel soyfoods products (Kitamura, 2002, personal communication).

Soybean and Soyfoods in Australia

Current Soyfoods Markets

Australia is multicultural with large ethnic and cultural minorities for whom soyfoods are a traditional part of the cuisine. From this traditional base and propelled by

the positive health aspects of soyfoods consumption, soyfoods are expanding into the general Australian community. Between 30 and 50% of the Australian soybean production is now used for direct human consumption. Principal uses include soymilk, tofu, tempeh, and soy flour as a bread improver. The market for soymilk in particular is expanding dramatically (~30% annually), and there is a major battle underway for market share between the whole-bean and the isolate-based (i.e., defatted and fortified) soymilk varieties. At this stage, whole-bean soymilk is winning because of its more healthful image.

Modern Soyfoods Cultivars

In Australia, breeding of soybean has focused on yield and disease resistance since its inception in the 1950s (45). Somewhat serendipitously, the cultivars Dragon and Bowyer were released in the 1970s and found to be acceptable for the production of tofu and soymilk (Table 14.8). These cultivars are still in use today, because subsequent releases have largely failed to achieve advances in functional quality over these cultivars. However, efforts are underway to develop cultivars with improved soyfoods properties as well as higher yield and increased disease resistance (Table 14.9). Table 14.10 lists some of the desired breeding traits for traditional soyfoods cultivars. Recently, research has focused on understanding the variation in food processing attributes of locally adapted breeding material and on introducing extra variation for these traits, as required, using cultivars from Asia as parents (46). Incorporation of cultivars from Asia as parents in the breeding program is more difficult than selection from within the adapted Australian material, because most Asian cultivars have extreme susceptibility to the foliar disease bacterial pustule (caused by *Xanthomonas campestris* pv. *Glycines*) and susceptibility to pod shattering (dehiscence) at maturity.

TABLE 14.8
Cultivars Used for Soyfood Purposes in Australia

Variety	U.S. maturity group	Specialty attribute or use	Parentage
Djakyl	III	Flour	Banjalong × DHF 5
Curringa	IV	Tofu	Unknown Japanese parent × HF
Bowyer	IV	High tofu quality	Williams × Beeson
791	V	Makes very white soymilk	Gasoy × Tracy
A6785	VI	Low gelling, good for soymilk	Young × D74-7741
Centaur	VI	Tofu	Davis × Bragg
Melrose	VI	High isoflavone content	HC78-676BC (2) × ATF 8
Dragon	VII	Tofu	Davis × Bragg
Jabiru	VII	Flour	From a recurrent selection population ^a
Manark	VII	Flour	Davis × Bragg
Warrigal	VII	Flour	Davis × Nessen

^aDerived from Davis, Flegler, Canapolis, BK 1445, P 24, Williams, Chung Hsien No. 2, Taichung 4, PI 200492, E.G.I., Aki Sengokku, UVF 72-1, 70/39, 62-2-6-3-B1, SH1188, Fitzroy, and HS 1421.

TABLE 14.9

Cultivars of Asian Origin Currently Being Employed in Soyfood Breeding in Australia

Variety	U.S. maturity group	Specialty trait or use	Origin
<i>Glycine soja</i>	0	Small seed size	China
He Dian 22	I	Tofu quality and high protein	China
Kaohsiung #1	I	Tofu/edamame quality	Taiwan
Shirome Diazu	I	Tofu quality	Japan
Toyomasari	I	Tofu quality, thick seed coat	Japan
Enrei	IV	Tofu quality, 11S subunit	Japan
BC KS #10	V	Ix ₁ , Ix ₂ , Ix ₃ alleles	Taiwan
Jizuka	V	Natto quality	Japan
Suzuyutaka	V	Tofu quality	Japan
Tachiyutaka	V	Tofu quality	Japan
Yomeyutaka	V	Tofu quality and Ix ₂ , Ix ₃ alleles	Japan
G2120	VIII	Small seed size	Indonesia

Many Japanese soyfoods cultivars also carry alleles for photoperiod response not found within adapted Australian cultivars, with the result that progeny from Japanese × Australian cultivars segregate widely for maturity. Selection for the above-mentioned traits necessitates larger breeding populations for soyfoods breeding than for commodity breeding in which parents are more adapted to Australia. A benefit of using Asian soyfoods cultivars in Australian breeding is that many have resistance to soybean mosaic virus and phytophthora root rot caused by *Phytophthora sojae*.

Breeding for the Soyfoods Market

Previous sections of this chapter summarized the soyfoods market and soyfoods cultivar development for China, Japan, the United States, and Australia. In the present section, underlying factors that affect breeding strategy and selection targets are reviewed for specific soyfoods. Suggestions for breeding targets, when they are offered (e.g., [Table 14.10](#)), should be taken as guidelines rather than as absolute requirements for the following reason: Although buyers tend to have some agreement about the nature of an ideal soyfoods cultivar, soyfoods processors are not uniform in their requirements, and their standards can vary from year to year. Variation in acceptance of beans for the soyfoods market often has to do with price and availability of seed in a given year. In that regard, acceptability of a less-than-perfect cultivar usually improves as its market price decreases. The exact cutoff in quality below which a company will not go varies with the availability of high-quality seed. High-quality seed can be blended with lower-quality seed sources to extend the natto bean supply.

Tofu

Tofu is a curd made by coagulation of the protein and oil in soymilk (47). The two main types are silken, or soft, tofu and momen, or hard, tofu. The main difference between

the two types is that silken tofu is formed through the coagulation of soymilk to form a curd. Momen tofu undergoes the extra step of pressing the curd to remove more liquid or whey, and results in a firmer curd. The degree of desired firmness varies with manufacturer and market preferences. There is wide variation in the basic procedure used to make tofu, but the key steps are (a) soaking the beans and grinding them into a slurry with water; (b) cooking the soybean slurry to form soymilk; (c) adding a coagulant, most commonly magnesium chloride, calcium sulfate, or glucono-D-lactone (which may be used pure or in combinations to achieve different flavor or textural characteristics); and usually (d) heating to facilitate coagulation. Silken tofu is often coagulated in the container in which it is to be sold. In the momen tofu-making process, the curd is pressed to remove moisture and form a cake.

The yield of tofu can be defined as the weight of fresh tofu produced from a unit of harvested soybean. There is strong evidence that choice of cultivar influences the yield and quality of tofu (12–15,48–53). The soybean breeder is therefore in a position to make significant changes to the tofu-making potential of soybean through selection. The main traits that the breeder needs to consider are protein and sugar content, seed size, hilum color, gelling properties, and tofu color. (Table 14.10). Genetic selection for these traits and their relation to tofu yield and dry-matter solubility are discussed in the following sections. Environmentally influenced variation in these traits is substantial and is also discussed in the context of breeding protocol.

TABLE 14.10
Desired Breeding Traits for Traditional Soyfood Cultivars

Soyfood	Desired breeding traits ^a
Tofu and soymilk	Yellow seedcoat with yellow or light hilum 100-seed weight 18–22 g Protein content > 45% Oil content > 20% Sugar content > 8% 11S/7S = ? Null lipoxigenase ?
Natto	Yellow seed coat with yellow hilum 100-seed weight < 9 g Hard seed < 0.5% Sugar content > 10%
Edamame, maodou	Green or yellow seedcoat or green cotyledon acceptable Mature 100-seed weight > 25 g Few or no one-seeded pods Pods with sparse gray pubescence Sucrose > 10%
Sprout	Soft texture Yellow seedcoat 100-seed weight < 15 g

^aAlthough buyers tend to have some agreement about the nature of an ideal soyfood cultivar, soyfood processors are not completely uniform in their requirements and their standards can vary from year to year.

Environmental Influences on Tofu Yield and Solubility of Seed Dry Matter. It is important to note that there is substantial year-to-year variation in tofu quality and yield from a single cultivar (54). In many cases, environmental variation for tofu yield may be greater than the genetic variation under investigation by the breeder. Therefore, a breeder must be prepared to overcome substantial environmental influences in the formulation of screening and testing methods. A standard breeding procedure for coping with large environmental effects (recommended by the authors) is to practice selection only among genotypes grown in common environments and of similar maturity, and to always include a standard soyfoods cultivar for comparison.

Post-harvest quality and conditioning of soybean also appear to have greater effects on tofu yield and solubility of seed dry matter than does cultivar choice. Thus, excellent seed handling protocol and prompt testing after harvest is important if one is to make the best comparisons of cultivars and breeding lines for tofu yield. In that regard, a main component that affects the yield of tofu is solubility of seed dry matter in the soymilk phase (or intermediate phase) of tofu making (53). Seed dry-matter solubility can vary substantially due to storage conditions of the beans (55). Fresh undamaged beans have a higher proportion of the seed dry matter recovered in the soymilk, a higher absorbance of water during soaking, and a higher tofu yield (54). Lowered solubility occurs principally when beans have been stored improperly at high temperature and humidity. Poor solubility can also occur when beans are stored at very low moisture content (56,57). Poor storage may also reduce the coagulative properties of protein after it has been successfully solubilized from the bean (56). Lowered solubility and poorer coagulation may also occur with cracked or split soybeans, even when relatively freshly harvested (55). A practical guideline to follow is that any factor that lowers germination also reduces tofu yield.

Genotypic Effects on Tofu Yield. Typically, genotypes with greater seed protein content produce a greater yield of tofu (38,53,58). Although the genetics of tofu yield are not clear, at present, qualitative genetic analysis suggest that tofu yield is controlled by at least one major gene plus modifiers. Heritability values for dried tofu yield have been as high as 85% in some crosses, with the major gene accounting for approximately one-half of the heritability (58). These results suggest that both the major genes and modifiers are sufficiently important to be utilized in breeding (58). Fresh tofu yield correlates positively with 100-seed weight and seed protein content, and negatively with seed oil content. Dried tofu yield correlates positively with the recovery of carbohydrate, oil, and protein from the seed (13,14). Oil content of fresh tofu correlates positively with seed oil content, although protein content of fresh tofu is not closely related to seed protein and oil content (59). Taira (38) also reported a positive relation between seed size and tofu yield.

Cultivars with larger seeds that approach spherical shape generally have greater soluble dry matter (and hence greater tofu yield) than those with small seeds (60), because of their more favorable surface-to-volume ratio, which reduces the amount of seedcoat present. The seedcoat is largely insoluble and is a minor component of finished tofu (57). For genotypes that attain approximately 20 g per 100 mature

seeds, the seedcoat comprises only about 5–7% of the weight of the seed. For seeds larger than 20 g per 100, other seed traits may have a greater effect on dry-matter solubility than further increases in seed size (57).

An additional restriction on seed size for tofu is that a 100-seed weight of 25 g or greater is associated with approximately 8% or more reduction in seed yield in the field (61). These factors have led to the acceptability of cultivars with 100-seed weight of 18–22 g for tofu manufacture.

Seed Protein and Gelling Properties of Tofu. Consumer preference for degree of tofu firmness can vary with culture and personal taste. Tofu firmness can be affected substantially by tofu manufacturing method, choice of soybean genotype, and crop harvest conditions. Much of the underlying basis for genetic variation in tofu firmness is in the differential properties of globulin storage proteins in the seed. In general, soybean storage protein is composed of three main fractions defined by sedimentation value as: 2S (α -conglycinin), 7S (β -conglycinin), and 11S (glycinin). The 2S fraction typically contains proteins such as protease inhibitors (62–64); the 7S fraction is composed of trimers of α , α' , and β subunits (65,66); and the 11S fraction is composed of hexamers of various acidic and basic subunits (67–69). The assembly, structure, and nature of the genes that encode these proteins are well documented (70–72). The 7S and 11S fractions account for about 70% of the total seed protein (73–75). The content of glycinin expressed as a percent of total protein and total dry seed weight varies among cultivars from 31.4 to 38.3% and from 13.5 to 17.8%, respectively (52,74,76–83).

Soy curd made from crude 11S is significantly harder than that made from crude 7S, and springiness and cohesiveness are slightly higher in soy curd with a higher proportion of 11S (83). The ratio of 11S to 7S globulin proteins in the seed also affects gelling characteristics and texture of tofu (40,74,79). [Table 14.11](#) lists the 11S-to-7S ratios of some varieties of soybean cultivars. The 11S-to-7S ratio is reported to range from 0.3 to 4.9 (82). The general trend is that beans with a high 11S-to-7S ratio make harder, higher-yielding tofu than those with a low ratio. However, not all genotypes with high 11S-to-7S ratios produce the same firmness. The gelling potential of 11S protein varies among cultivars because it is made up of many subunits with differing gelling characteristics (84,85). In contrast, a low 11S-to-7S ratio results in consistently poorer gelling characteristics because of the greater uniformity of gelling response in 7S globulins (56). The ratio of 11S to 7S proteins and the makeup of the 11S globulins therefore account for some of the genotypic differences in tofu texture and quality made from beans of similar seed protein content (52). The 11S-to-7S protein ratio in soymilk and soy curd is correlated with that in the seed (52). The environmental effect on the 11S-to-7S ratio may be larger than the genetic effect (86).

Seed Color. Beans with a yellow or light-buff hilum and light-yellow seedcoat are preferred for tofu manufacture. Although the color of tofu appears to be independent of hilum color, and to some extent of seedcoat color (54), any pieces of

TABLE 14.11

Ratio of 11S to 7S Proteins in Seeds of Soybean Cultivars

Variety	Origin	11S-to-7S ratio	Reference
Clark	USA	0.90	Wolf <i>et al.</i> , 1961 (77)
Hakuhou		0.50	Wolf <i>et al.</i> , 1961 (77)
Clark and Hawkeye	USA	0.84	Wolf <i>et al.</i> , 1962 (78)
Hakuho	Japan	0.78	Saio <i>et al.</i> , 1969 (74)
Akasaya	Japan	0.83	Saio <i>et al.</i> , 1969 (74)
Aobata	Japan	0.68	Saio <i>et al.</i> , 1969 (74)
Norin	Japan	0.77	Saio <i>et al.</i> , 1969 (74)
Shirotsurunoko	Japan	0.57	Saio <i>et al.</i> , 1969 (74)
Shofuku	Japan	0.86	Saio <i>et al.</i> , 1969 (74)
Suzuyutaka	Japan	1.55	Kitamura, 1995 (79)
Tachiyutaka	Japan	2.24	Kitamura, 1995 (79)
Karikei 434	Japan	5.88	Kitamura, 1995 (79)
E line	Japan	3.75	Kitamura, 1995 (79)
Proto, Vinton, Sturdy	USA	1.6–3.2	Ji <i>et al.</i> , 1999 (80)
213 <i>G. soja</i> accessions		0.36–4.40	Xu <i>et al.</i> , 1990 (81)
1,000 soybean accessions		0.3–4.9	Harada and Hossain, 1991 (82)
13 soybean varieties (52)		1.60–2.51	Cai and Chang, 1999
Soybean varieties		1.29–1.38	Kim <i>et al.</i> , 1995 (83)

dark-pigmented hilum or seedcoat that are not removed during the making of soymilk will appear as an unsightly contaminant in tofu. It is possible, however, to make excellent-quality tofu from dark hilum beans if the beans are dehulled prior to use or if the soymilk is carefully filtered. To minimize the number of steps in tofu preparation (i.e., to avoid dehulling, etc.), manufacturers prefer cultivars with clear or light-buff hila for use in tofu manufacture.

Color of tofu appears to be affected by choice of cultivar (51,60), environmental conditions during seed production (53), and storage conditions after harvest. Yellowness is considered unattractive and is associated with aging of tofu products and off-flavors. Beans that produce yellow pigments in tofu are therefore undesirable for consumers, who will tend to assume that tofu made from these beans is stale. Fortunately, there is substantial genotypic variation for color (51,53). To ensure that all shipments to a processor meet minimum quality specifications for traits such as color, a trading company or grain distributor may blend seed lots of higher and lower quality prior to shipping. Different sources may include seed from several different cultivars or perhaps multiple seed lots of the same cultivar, all with clear hilum and large seed size (54). Green discoloration of the cotyledon from premature harvest will be passed on to the final tofu product, as will other pigments.

Brown pigments on the seed, known as seed, mottling or bleeding hilum, are undesirable and have become an increasingly severe problem for U.S.-grown tofu-type

cultivars in recent years. Several U.S. cultivars intended for the tofu market have been discontinued for this reason. The problem is likely the result of soybean mosaic or bean pod mottle viruses, but factors influencing the recent severity of the problem have not been determined. Chilling temperature at flowering has been shown to increase pigmentation of the hilum as well as seedcoat cracking (87–90). Cultivars carrying the I allele related to the yellow-hilum trait may be more susceptible to seed mottling than other types (R.L. Bernard, University of Illinois, 2002, personal communication)

Sugar Content. Soluble sugars in soybean seeds are important for the flavor of tofu (38), although the quantity of sugars remaining in the tofu varies with type and manufacturing process. Free sugar content is especially important in Kinugoshi tofu and packed tofu, which contain a large amount of whey. Approximately 12% of the seed dry weight is nonstructural carbohydrate at physiological maturity. Starch typically accounts for 1–3% of the seed dry weight (91). The majority of the carbohydrate at seed maturity is either sucrose (41–68%), stachyose (12–35%), or raffinose (5–16%). Sugars can easily leach from tofu when whey is removed during pressing. There is a strong negative genotypic correlation between protein content and sugar content in seed (92–95) and breeders need to be careful not to lose too much sugar content when selecting for higher protein content for tofu.

Undesirable Flavors in Tofu. Undesirable flavors in tofu include the grassy-beany taste generated by lipoxygenase when it oxidizes fats, and the astringent tastes and texture of the isoflavones and saponins in soybean. Oxidation of fats can be avoided by grinding the soybeans in water at a temperature greater than 70°C. However, this has the disadvantage of reducing protein solubility and hence yield of tofu (56). In places where tofu is being manufactured for traditional consumers, a higher level of beany flavor is accepted compared with places where tofu consumption is a more recent trend. Increased isoflavone and saponin content may be associated with undesirable flavors. Although such relationships are not well documented, breeding lines developed for edamame and selected for desirable taste by USDA breeder Kuel Hinson were also unusually low in isoflavone content (A. Blount, University of Florida, 2003, personal communication). Beans with reduced lipoxygenase can be bred (39,79), and beans with low isoflavone and saponin content can be selected (96–98). However, the positive marketing appeal of enhanced isoflavone content for improved health overrides the negative taste aspects in some market segments.

Natto

Natto is a traditional fermented food product originating in Japan and made through the fermentation of whole beans by the bacterium *Bacillus natto*. A good-quality natto product should have uniformly small seeds, be light in color, and be covered

with light-colored mucilage. It should have the traditional aroma and flavor and a soft texture. Recently, some manufacturers have introduced natto with low aroma to the market using altered strains of *B. natto*, in response to changing consumer preferences (99,100). When mixed using chopsticks, the mucilage covering the natto should lighten in color and the beans should cling together in a manner permitting easy transfer to the mouth. It is desirable that long strings of silk-like mucilage should connect a separated natto morsel to the main dish. Natto should have a minimum of broken beans and a low content of ammonia. Natto is consumed straight from the refrigerator after mixing with a small quantity of soy or fish sauce, sometimes with the addition of finely sliced spring onion, seaweed, or mustard. It is served either as a side dish with steamed rice or placed directly on rice. Manufacture of natto includes the basic steps of cleaning the soybean seeds, soaking, removal of hard seeds, rinsing, steaming, inoculation with *B. natto*, and fermentation.

A first requirement for a natto variety is small seed size. Manufacturers prefer a near-spherical seed of smaller than 9 g per 100 seeds, which should fall though a screen with a 5.5 mm (or 14¹/₂/64-inch) diameter round hole (Table 14.10) (47,93,101–103). Near-spherical seeds rather than those with a flatter profile are preferred simply to reduce the ratio of the tough seedcoat to softer cotyledon. A second important requirement is soft texture. A softer natto product can usually be obtained from seeds with a higher content of soluble sugars (93). A minimum total sugar content of 10% is usually required in the mature seed of a natto cultivar. A relatively low sucrose content with high stachyose and raffinose contents is considered to be favorable for maintaining uniform fermentation—sucrose for fast early digestion and oligosaccharides for later digestion (41). Rapid water absorbance during soaking also results in softer seeds in the finished product (38) and a higher yield of finished natto. Not all small-seeded cultivars absorb water at the same rate (104–106). Some natto manufacturers require that water uptake during seed soaking, the first step in natto production, meet a minimum standard. The American small-seeded cultivar Vance, for example, is less able to absorb water over a 12-hour time interval than are many other small-seeded or large-seeded types. For this reason, Vance can be used as a control or standard for selection of breeding lines with improved water uptake during soaking. Genotypic variation for cooking time after soaking has been noted in soybean (107) and cowpea (*Vigna unguiculata* L. Walp.) (108). Softer natto can also be achieved by increasing the steaming time during processing, but this adds additional manufacturing cost and may darken the color of the final natto product (109).

An additional requirement is that the color of the finished natto product should be yellow rather than brown. Color appears to be largely conditioned by the quality of the raw beans. Manufacturers prefer uniform light yellow colored seed with yellow hilum, though buff hilum is accepted. There is substantial cultivar variation for color of finished natto. This variation can be identified in the raw bean and in the finished natto product (93). Color appears to be independent of other quality attributes such as seed size, protein, oil or sugar content (93) and would therefore need to be measured independently when breeding for improved natto.

Viscosity of mucilage is also important and is increased with higher levels of bacterial development. Bacterial development is greater for batches of seed with higher sugar content and smaller seed size (110). Viscosity can also be increased by longer periods of steaming or an increase in fermentation time (109). However ammonia content also increases with increasing fermentation time.

Calcium content above about 2,500 mg/kg is anecdotally reported to adversely affect the fermentation process and is therefore considered undesirable. However, literature relating calcium and fermentation is sparse. Seed calcium content below 870 mg/kg appears to decrease germination substantially, and suggests that low calcium levels in the seed would also detrimentally affect natto quality (111). Soybean seeds typically contain 1,800 to 3,400 mg/kg of calcium at maturity (112–114).

Genetic aspects of natto taste and aroma are not well understood. The good flavor in natto is associated with the presence of glutamic acid, which is liberated from the soybean by protein hydrolysis during fermentation. The characteristic aroma of natto is said to be related to diacetyl production. Ammonia-related volatiles are considered very undesirable.

A selection strategy for natto cultivar development must consider both the effect that variation in seed quality might have on the final product and the effect that variation in processing technology can have on the quality of natto. The needs of manufacturers are paramount. Manufacturers are likely to prefer small spherical seed with high sugar content because these traits should result in the shortest manufacturing time, highest yield of natto, greatest mucilage production, and lowest ammonia content in the natto. There is genotypic variation for sugar content, but cultivars developed outside Japan and China appear to have generally lower sugar content (115). Small-seeded North American cultivars also tend to produce a “less soft” natto than traditional Japanese cultivars. The basis for this has not been determined at present, but may be related to sugar content as well as other factors. Genotypic differences in color are likely to be relatively consistent over time, leading to preferences by manufacturers for specific cultivars. As with tofu cultivars, the bleeding hilum trait has become a serious problem in the United States in recent years, and several U.S. natto cultivars have been discontinued for this reason.

Edamame *or* Maodou

Vegetable soybean is a traditional food of Japan and China that is now consumed throughout East Asia (116). Traditionally, the whole plant is harvested green at the R6 or R7 stage (117–119) and transported intact to market to assure customers of the freshness of the product. After purchase, pods are removed from the plant, boiled, and consumed as a snack food. The final product, boiled salted pods, should be blemish-free and bright green (17,120). Traditionally, cultivars with a genetically controlled “stay green” seedcoat and cotyledon have been preferred by growers because the harvest period can be extended closer to maturity of the plant without experiencing the yellowing associated with maturity. Seed pods

should have sparse gray pubescence and contain three seeds per pod, though two-seeded pods are acceptable in the market (121). There should be an absolute minimum presence of one-seeded pods because they require greater effort to shell and are therefore disliked by the consumer. Four seeds in a pod are not preferred because the number four is considered unlucky in Japanese culture. In recent times a reselection of the old Japanese cultivar Tanbaguro has become popular for *edamame* because of its exceptionally smooth texture, high sugar content, large seed size, and good flavor, in spite of it having a black seedcoat and stiff tawny pubescence on the pod (122).

Desirable *edamame* has very large seeds, high sugar levels, and a smooth texture (Table 14.10) (121). Cultivars suitable for *edamame* purposes generally possess greater than 10% dry weight of sucrose from mid-pod development until maturity (123). It is thought that the genetic removal of lipoxxygenases will result in a bean with less beany flavor and greater acceptability to the market (120). Young *et al.* (124) found that beans that were sweet were also somewhat nutty, less beany flavored, slightly oily, lacking an unpleasant aftertaste, and generally better in overall eating quality. This is not surprising given that sugar content is positively correlated with oil content and negatively correlated with protein content (92–95). For the fresh-frozen market, uniformity of maturity, a thicker pod wall to reduce freezing damage, and plant habit to permit mechanized harvest is required in addition to the quality traits required in the fresh product (125). Cultivar development for *edamame* for the fresh market should focus on production in multiple sequential planting dates so that the harvest period can be maximized.

Soymilk

There are two kinds of soymilk produced for the market. Traditional soymilk is made from whole beans in the same way as the first few steps of tofu manufacture (126–128). This soymilk contains nutrients, isoflavones, saponins, and other soluble components of the soybean from which the soymilk is made (129,130). Nontraditional soymilk is manufactured from soy protein isolate, to which fats, sugars, and carbohydrates are added to improve flavor and generate a nutritional profile similar to that of cow's milk. Some manufacturers add isoflavones back into the soymilk in order to make health claims about the product. Although globulin proteins that coagulate well are preferred for tofu, cultivars with globulin proteins that paste rather than gel are preferred for soymilk because such proteins are more likely to remain in solution (38).

Designing Future Soyfoods Cultivars

In addition to the application of transgenic approaches (131,132), several natural gene mutations have been discovered that enable genetic flexibility in tailoring soybean seed composition to enhance consumer preference for soyfoods products. This ability not only allows the manipulation of single genes that regulate the activity of

an enzyme in a particular metabolic pathway, but also the melding of functional combinations of genes to produce novel phenotypes. As gains are made in understanding of the genetic and biochemical mechanisms that govern synthesis of protein, oil, carbohydrate, and minor constituents, innovations in soybean seed composition may stimulate consumer demand for soyfoods products in a number of ways, ranging from new health claims for products that are “Low in Saturated Fat” or “High in Omega-3 Oils” to improved flavor and texture of traditional soyfoods. The overall effort has been to design seed composition for specific soyfoods products.

Increasing Protein and Oil Concentration

There is a wide range of genetic variation in protein (Fig. 14.2) and oil (Fig. 14.3) concentration among accessions of the USDA soybean germplasm collection (24). The reported range of protein concentration is 34.1–56.8% of seed dry mass, with a mean of 42.1%. Oil concentration among the accessions in the collection may range from 8.3–27.9%, with a mean of 19.5%. There generally is a negative correlation between protein and oil concentration in soybean (133). This means a genetic or environmental influence that causes an increase in protein often results in a decrease in oil. Thus, it is extremely rare to find germplasm in which the concentration for both

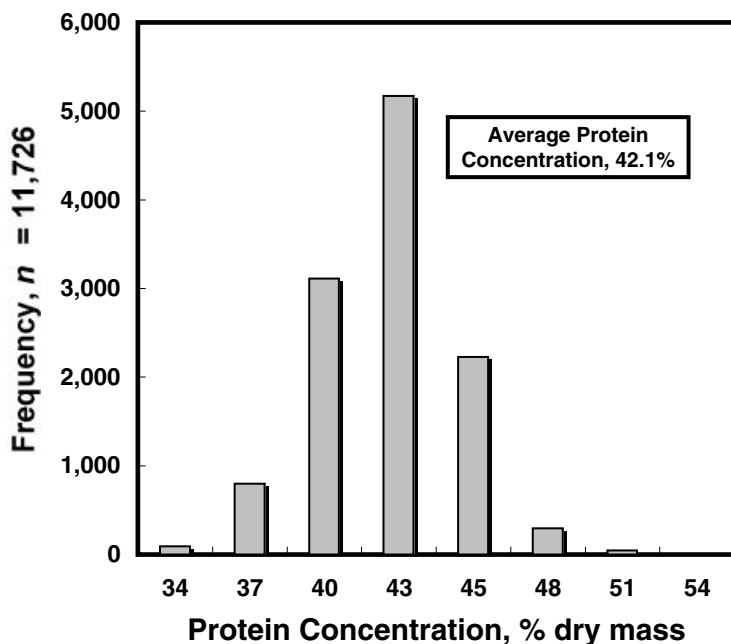


Figure 14.2. Distribution of portein concentration among accessions of the USDA soybean germplasm collection.

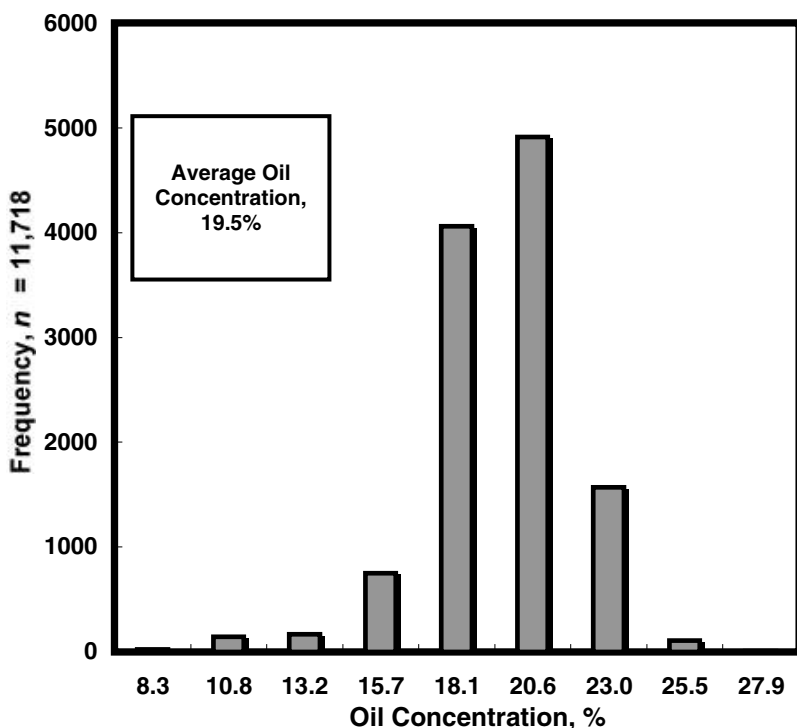


Figure 14.3. Distribution of oil concentration among accessions of the USDA soybean germplasm collection.

protein and oil is relatively high. There is also a negative genetic correlation between protein and yield (134). This relationship has significantly impeded commercial production of soybean with greater than average protein concentration. However, recent evidence suggests that genetic manipulation or combination of certain genes may enable higher than normal protein concentration in germplasm that maintains competitive levels of oil and yielding ability. Therefore, it is possible to overcome these barriers.

If, on average, soybean seed contains about 42.1% protein and 19.5% oil (dry mass), a practical target for improved soyfoods cultivars is about 44–45% protein and no less than 18% oil. Unfortunately, such a phenotype is not common among current commercial soybean cultivars, but this goal is attainable. Specialized breeding methods, such as recurrent-index selection, have been used to increase yield in a high-protein population (135,136). With this technique, a significant gain in yield may be achieved without losing the high-protein trait. Several agronomic high-protein cultivars have been developed in this manner. The prototype for this concept was the cultivar Prolina, which exhibited higher than normal protein concentration with minimal loss in oil concentration (137). Now agronomic high-protein lines are beginning to emerge, such as S96-2641 from the University of Missouri (S.C.

Anand, personal communication). These cultivars demonstrate that it is possible to break the negative genetic correlations and achieve simultaneous gains in protein, oil, and yield.

Soybean Protein Composition

Among all vegetable sources of protein, soybean may provide the most complete amino acid balance for human food and feed. However, soybean protein has less than optimal levels of some essential amino acids, such as methionine and cysteine. Therefore, improvements are needed to enhance soybean protein quality for the soyfoods market. In the United States, the primary goals for enhancing soybean protein quality are (a) to improve essential amino acid balance, and (b) to increase digestibility of the meal. Essential amino acid balance may be augmented by regulating the expression of genes in particular amino acid pathways or by increasing the concentration of total crude protein. Digestibility of soy protein can be improved by reducing the level of oligosaccharides (raffinose and stachyose) in soybean seed, which also may result in improved flavor from the increase in soluble sugars. An additional benefit may be gained from genetic traits that improve the functional characteristics of soy-protein. These attributes are needed to expand applications for all vegetable protein-based products, including soyfoods. It is also important to ensure that soy-based foods contain a desirable level of isoflavones, which may convey certain health benefits. Of course, these attributes must be effected in soybeans that have good yielding ability.

Potential for Altering Protein Composition. Many seed storage protein genes from soybean have been isolated, sequenced, and expressed in transgenic plants to gain a better understanding of their function and regulation. The potential of genetic engineering approaches to modify soybean protein composition is evident. However, control of gene copy number, the site of transgene insertion, and effects of amending the native primary structure of polypeptides pose interesting problems relating to the final level of expression and storage protein deposition. These concerns impede the achievement of objectives to elevate levels of 'limiting' essential amino acids. With the exception of the introduction of novel proteins from sources such as the Brazil nut (*Bertholletia excelsa* H.B.K.) (138–140), molecular genetic manipulation of specific genes that encode these storage proteins has not yielded significant or obvious changes in the concentration of essential amino acids such as methionine and lysine in soy protein (141). This result may be attributed to the complexity of the protein synthetic pathway, and to the effects of various environmental influences on the constituent enzyme systems. Yet, significant knowledge about the biological mechanisms that regulate protein composition has been gained from these studies, and future progress will be aided by the investigation of natural or induced mutations in the subject storage protein genes.

Mutations in 7S Storage-Protein Genes. As mentioned previously, β -conglycinin (7S protein) is composed of three different subunits, α , α' , and β . There are at least 15 members of the gene family that governs 7S protein synthesis. These β -conglycinin

genes are clustered in several regions of the soybean genome, and full-length sequences are highly homologous (142). Apparently, β -conglycinin gene expression is subject to both transcriptional and post-translational regulation (143). The gene sequence for the β subunit (144) and the α' subunit are known (145). Although the structure of the gene that encodes the α subunit has not been completely determined, it may be composed of six exons that have similar organization to that found in the α' subunit gene (146). When the α and α' subunits are suppressed by sequence-mediated gene silencing in transgenic soybean seed, no significant differences were detected in total protein content, but 11S protein content increased at the expense of 7S protein (147). Similar elevation in 11S protein content is detected in soybean varieties (with induced mutations) that lack the α and β subunits (148) or all three subunits (149). Given that 11S proteins are enriched in sulfur-containing amino acids compared to 7S proteins, the higher 11S-to-7S ratio in these germplasms should influence amino acid composition in a favorable manner. However, the individual concentrations of methionine, cysteine, and lysine in soybean seed with low β -conglycinin levels was only marginally greater than those in ordinary cultivars (150). Hence, more exacting methods may be required to detect the effect of mutations in storage protein genes on amino acid composition. As an example, comparison of amino acid residues per mole of purified 11S and 7S proteins from the high-protein line Prolina and the high-oil line Dare revealed a significant increase from 1 to 5 cysteine residues per mole of 7S protein in the high-protein line (151,152).

Mutations in 11S Storage Protein Genes. The glycinin gene family encoding 11S subunits of soybean storage protein is composed of at least five (Gy_1 to Gy_5) gene members (71). The inheritance and organization of the glycinin gene members has been documented extensively (153–155). The products of these major glycinin genes have been classified into two major subunit groups based on their sequence homologies. Group I contains $A_{1a}B_{1b}$, A_2B_{1a} , and $A_{1b}B_2$ subunits. Group II contains $A_3A_4B_3$ and A_3B_4 subunits (153). Gene sequences have been reported for Gy_1 (156), Gy_2 and Gy_3 (157), and Gy_4 (158). Several of these genes have been mapped to positions in the soybean genome (159,160). Natural aberrations occur in these genes, such as the recessive Gy_3 allele in the cultivar Forrest (161).

Influence of Nutrition on Storage Protein Gene Expression. Transgenic manipulation of regulatory steps in the synthesis of the amino acids (methionine, cysteine, lysine, threonine, and isoleucine) derived from aspartic acid may lead to increased accumulation of free threonine or lysine, but such events apparently do not elevate the level of these two amino acids in storage proteins. Yet, normal soybeans grown with varied levels of nutrients, such as sulfate, do exhibit significant changes in the amount of methionine and cysteine, particularly in sulfur-rich proteins, which likely occur in the 2S protein fraction (155). The elevated expression of such proteins has a pronounced effect on the normal complement of 7S and 11S proteins. For

example, when sulfur is limiting, seeds typically contain lower levels of glycinin, and greater amounts of the β subunit of β -conglycinin (162). The latter effect is mediated by up-regulation of transcription of the *Cgy₃* gene that encodes the β subunit of 7S protein. Application of nitrate to nitrogen-deficient soybean may elicit a similar response resulting in an elevation of mRNA for the β subunit (163). Concomitant effects may be observed in the expression of the *Cgy₂* gene (α' subunit), which is linked (in terms of trait inheritance) to the *Cgy₃* gene (164,165). These observations demonstrate that the supply and balance of nitrogen (N) and sulfur (S) nutrients exert regulatory effects on the relative abundance of specific soybean storage proteins (166,167).

In general, increased supply of N and S nutrients not only effects an increase in total protein, but also may influence the patterns of 11S and 7S protein accumulation in developing seeds (168). The gain in protein content in response to increased N fertilization may be attributed to positive effects on the accumulation of both 7S and 11S proteins (168). This result is related to nutrient effects on transcriptional regulation of *Gly* and *Cgy* genes, which are up-regulated by high-N nutrition.

Association with Protein Functionality. Functional qualities inherent in plant proteins often limit their utility in soymilk and vegetable-protein food formulations (169). Compared to egg white albumin and casein, protein from commercial soybean cultivars has major limitations in solubility, water absorption/binding, and viscosity. These properties are determined by size, flexibility, and the three-dimensional conformation of the protein molecules. An elegant experiment (84) has demonstrated the impact of altered 7S and 11S content on the gelation properties of soymilk prepared from a low- β -conglycinin soybean line lacking α and α' subunits and from a low-glycinin soybean line lacking various 11S subunit groups (I, IIa, IIb, I + IIa, I + IIb, or IIa + IIb). The induced genetic mutations in these genes enabled significant variation in the 11S-to-7S ratio (from 3.8 to 0.1) in the soymilk treatments. Results showed that protein gel strength from low- β -conglycinin soybean (greater 11S protein) was about fourfold greater than that in low-glycinin soybean (greater 7S protein). Thus, there was a strong positive relationship between protein gel strength and the 11S-to-7S ratio.

In addition, it has been shown that protein functionality or its physiochemical properties may be influenced by the number of disulfide bridges between cysteine residues in the 11S and 7S proteins (151). In this case, the cultivars Prolina and Dare had the same number of cysteine residues per mole of 11S protein, but Prolina exhibited a fivefold increase in cysteine residues per mole of purified 7S protein compared to Dare. As is typical of conventional soybean, both 11S and 7S proteins purified from the cultivar Dare exhibited soft or poor heat-induced gelation properties. Similar results were found for the gelation properties of purified 11S protein from the cultivar Prolina; 11S proteins from both Prolina and Dare formed very soft gels that collapsed upon storage overnight, and purified 7S protein from Dare did not form a gel. However, purified 7S protein from Prolina became very viscous upon

solubilization in buffer and formed a firm gel that was strong enough for shear stress and strain tests. Therefore, the gelation property of 7S protein from Prolina may be attributable to greater hydrogen bonding among the constituent proteins. Hence, subtle variation in the primary structure of 11S and 7S subunits may be equally effective in enhancing the functional properties of soybean protein.

Soybean Carbohydrate Composition

Assuming total extraction of protein and oil, carbohydrate accounts for approximately 86% of the residual dry mass of mature soybean seed. The primary constituents are starch, sucrose and other soluble sugars, and oligosaccharides (raffinose and stachyose). As shown in electron micrographs (170) and chemical analyses (171,172), starch is the predominate carbohydrate early in seed development. Starch deposition peaks near mid-pod fill, then declines, and is nearly absent in mature seed. In conjunction with starch hydrolysis, soluble sugars (sucrose, fructose, and glucose) begin to accumulate prior to mid-pod fill as a function of elevated invertase and sucrose synthase activity (173). Raffinose and stachyose accumulate later in seed development (174). Typical ranges reported for mature seed are 41–67% sucrose, 5–16% raffinose, and 12–35% stachyose, as a percentage of total soluble carbohydrates (24).

Genetic Regulation of Oligosaccharide Content. As research progress continues to fine-tune soyfoods quality, attention will turn to reduction of the complex carbohydrates, raffinose and stachyose. The primary enzyme activities in the oligosaccharide synthetic pathway (Fig. 14.4) are galactinol synthase, raffinose synthase, and stachyose synthase (175,176). Genetic variation in complex sugar composition among strains of soybean suggests natural mutations in the genes that encode these synthases (177–181). Indeed, recessive alleles have been identified at *Stc-1* loci that presumably reduce the activity of each enzyme (182). Two of these natural gene mutations mediate reduced raffinose synthase activity; the third recessive allele apparently causes lower galactinol synthase activity. The combination of all three recessive alleles has been shown to eliminate at least 97% of the normal levels of raffinose plus stachyose in soybean seed, with concomitant increase in sucrose (Table 14.12). Unfortunately, these low-stachyose beans reportedly suffer from poor seed germination (183–185). This problem has impeded the use of these valuable traits in commercial cultivar development.

Soybean Fatty Acid Composition

Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) are the predominant fatty acids of soybean oil.

Molecular genetic technologies have provided new insight into the biological mechanisms that govern fatty acid composition in soybean. Considerable information has been gathered from DNA sequences of nearly every gene that encodes an enzyme in the fatty acid synthetic pathway (186). These advances in knowledge have

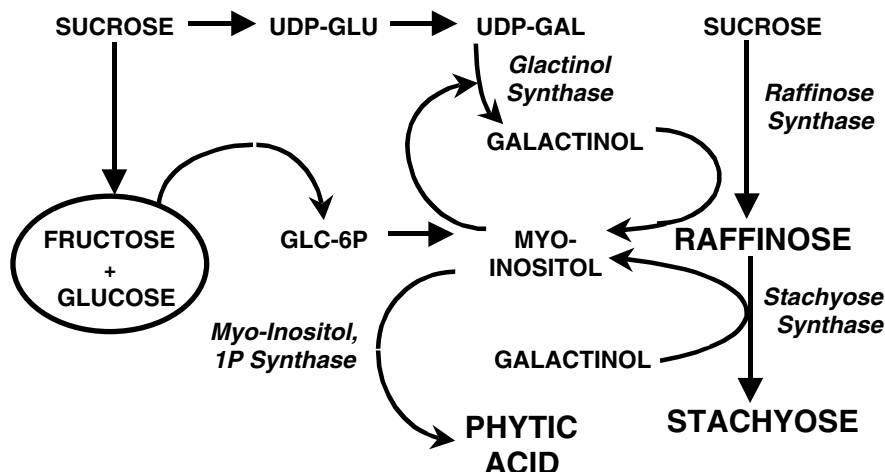


Figure 14.4. Diagram of the stachyose and phytic acid synthetic pathways in soybean.

TABLE 14.12

Genetic Manipulation of Soluble Carbohydrate Concentration in Soybeans

Mutations	% of total soluble carbohydrate		
	Stachyose	Raffinose	Sucrose
Normal ^a	43.5	9.3	47.2
Galactinol synthase	16.9	5.2	77.9
Galactinol + raffinose synthases	6.6	1.3	92.1
Galactinol + myoinositol-1P synthases	0.0	0.9	99.1

^aTotal soluble carbohydrate 7–12% of seed dry mass.

led to directed genetic modification of soybean oil composition (187) and better understanding of the functional structure of enzymes, such as acyl desaturases (188). Significant progress has also been made in the development of molecular genetic markers that facilitate the identification of genotypes in populations segregating for fatty acid traits, and the positioning of these genes on genetic maps of the soybean genome (189,190). However, the foundation for all of this technology rests upon the discovery or creation of natural mutations in genes that mediate altered oil phenotypes. These genetic resources are being used to determine the inheritance of traits and to transfer desirable genes to agronomic cultivars.

Genetic Modification to Reduce Saturated Fatty Acid Composition. N79-2077-12 was the first soybean germplasm released with reduced C16:0 concentration (191,192), and is the only known germplasm that carries a serendipitous natural

mutation, designated as the recessive *fap_{nc}* allele. Other soybean germplasm exhibiting about half of the C16:0 levels found in normal soybean oil have been induced with chemical mutagens such as ethylmethanesulfonate (EMS). These germplasm varieties may carry a combination of alleles: C1726 carries the homozygous recessive *fap₁* allele (193); A22 carries the *fap₃* allele (194); and ELLP2 carries the allele with temporary designation *fap** (195). Combinations of homozygous *fap₁* and *fap₃* (196) or *fap₁* and *fap_{nc}* (197) or *fap₁* and *fap** (195) alleles reportedly constitute transgressive segregates, from mating of the respective parental lines, that exhibit less than 4.5% C16:0. The inbred lines C1943 (with northern maturity) and N94-2575 (with southern maturity) are examples of selections in which *fap₁* and *fap_{nc}* are combined (198). Based on this information, it is highly probable that the mutations represented by the *fap₃*, *fap_{nc}*, and *fap** descriptors are different and distinct from *fap₁*. However, it is not known whether *fap₃*, *fap_{nc}*, and *fap** are independent or allelic to each other.

Given that *fap_{nc}* and *fap₁* segregate as independent loci, efforts have been made to identify the enzyme(s) they encode. Both of these alleles effect reduced C16:0-ACP TE activity (199). Genetic effects on the activity of this enzyme were also apparent at the transcriptional level. In addition, the mutation in the *fap_{nc}* allele is a natural gene deletion; and that *fap₁* represented a point mutation, where leucine was substituted for tryptophan at residue 140 in the C16:0-ACP TE primary structure (Wilson *et al.*, unpublished data). However, the function of other *fap* alleles has not yet been determined.

Genetic Modification to Alter Unsaturated Fatty Acid Composition. Soybeans typically contain about 24.2% C18:1 (24). The germplasm N78-2245 was perhaps the first soybean developed with higher levels (about 42%) of C18:1. This phenotype is attributed to a natural mutation in the FAD2-1 gene that encodes the predominant omega-6 desaturase in soybean seed. When a normal FAD2 gene is expressed in antisense orientation (or by cosuppression) in transgenic soybean, the seed oil may contain up to 80% C18:1 (187). Therefore, it may be presumed that natural mutations at *Fad* gene loci determine the high-C18:1 trait in nontransgenic soybean. Until recently, transgenic events appeared to be the only feasible approach to achieve soybean oil with exceptionally high levels of C18:1. However, through natural gene recombination, J.W. Burton (USDA-ARS at Raleigh, N.C.) has developed a population with segregates that range from 45% to 70% C18:1. An experimental inbred line (200), N98-4445, containing about 60% C18:1 has been selected from this population. It is believed that this line contains mutations that affect the product of two different isoforms of the FAD2-1 gene, which encodes the predominant omega-6 desaturase in soybean seed (R.E. Dewey, North Carolina State University, Raleigh, personal communication). Apparently, this natural mutation confers the high-C18:1 trait without the deficiencies in plant germination that are attributed to transgenically derived high-C18:1 germplasm (201).

N78-2245 (202) also exhibited lower C18:3 concentration. Other low-C18:3 germplasm strains have been developed through chemical mutagenesis. Wilcox *et al.*

(203) mutagenized the cultivar Century with EMS and selected a line, C1640, that contained about 3.5% C18:3. Inheritance studies revealed that this trait was controlled by a single recessive allele, designated *fan* (204). Hawkins, *et al.* (205) mutagenized the line FA9525 and selected a line, A5, that contained about 4% C18:3. The single recessive allele in A5 was designated *fan*₁. Subsequently, two additional mutations were described, *fan*₂ and *fan*₃, at *Fan* loci. When combined in the germplasm line A29, these alleles reportedly produce soybean oil with 1.1% C18:3 (206). In addition, two low-C18:3 plant introductions from the USDA's soybean germplasm collection—PI123440, identified by C.A. Brim (207), and PI361088B, identified by Rennie *et al.* (208)—contained natural mutations at *Fan* loci that were shown to be either allelic or identical to the original *fan* allele in C1640 (209). All of these respective *fan* alleles represent mutations in different genes or different mutations in the same gene, and the product of these genes is presumed to be the predominant omega-3 desaturase in soybean seed.

Influence of Multiple Gene Combinations on Oil Composition. The genetic resources documented above represent a positive avenue toward improved soybean oil quality. Such innovations must involve the combination of multiple gene mutations to produce commercial products acceptable to consumers. At this time, soybean oil with a low C16:0 and a low C18:3 concentration will be an initial step in the commercial process to improve soybean oil quality. A number of agronomic low-C16:0 plus low-C18:3 soybean cultivars are being developed that are adapted to respective areas of the entire U.S. soybean production region (maturity groups I through VIII). The first of these new cultivars is the maturity group V cultivar Satellite (200). The next improvement in oil quality for general-purpose applications involves transfer of the mid-C18:1 trait from germplasm such as N98-4445 to cultivars like Satellite. N98-4445 (derived from N97-3363-4) represents the only known mid-C18:1 soybean in the public sector.

Tocopherols and Isoflavones in Soybean Seed

Soybean contains several highly valued minor constituents, such as tocopherols and isoflavones. Soybean is the predominant commercial source of α -tocopherol (natural vitamin E). The isoflavones, principally diadzein and genistein, are physiologically active components of soybean meal. It is believed that isoflavones possess antioxidant properties, and that these properties are associated with a number of health benefits.

Tocopherols. Soybean oil typically contains three primary types of tocopherol: delta (2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-; gamma (2,7,8-dimethyl-2-(4,8,12-trimethyltridecyl)-; and alpha (2,5,7,8-dimethyl-2-(4,8,12-trimethyltridecyl)-tocopherol (210). In decreasing order, the relative effectiveness of these compounds as anti-oxidants is δ -, γ -, and α -tocopherol (211). Soybean contains a considerable amount of total tocopherols (ca. 1,000 to 2,000 ppm). However, genetically modified oils have been shown to exhibit significant changes in tocopherol composition (212–214). As an example, there is a positive correlation between γ -tocopherol and C18:3

concentration in the oil of mature soybean (Fig. 14.5). Thus, lower γ -tocopherol concentration may be expected in cultivars having genetically reduced levels of C18:3. By the same token, low-C18:3 soybean oils exhibited elevated levels of α -tocopherol or vitamin E. The apparent enrichment of total tocopherol, when measured by α -tocopherol, was a function of loss of γ -tocopherol. Therefore, soybean cultivars exhibiting a low-C18:3 oil should contain more α -tocopherol, and enriched amounts of extractable vitamin E should provide an additional beneficial aspect of genetic approaches to improve soybean oil quality.

Isoflavones. Soybean flavonoids exist as free aglycones or glycoside derivatives. The fundamental aglycone compounds are diadzein, genistein, and glycitein. These compounds are believed to contribute the physiological activities that are attributed to isoflavones (215). The glycosides (diadzin, genistin, and glycitin) may also occur as 6''-O-malonyl or 6''-O-acetyl derivatives of the three fundamental aglycones. Total

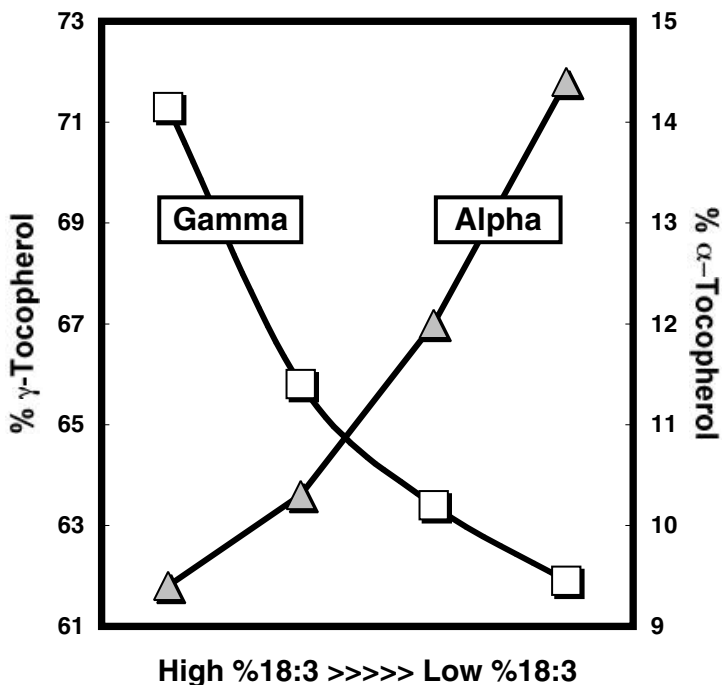


Figure 14.5. Relation of tocopherol concentrations to C18:3 concentration in mature seed of soybean germplasm with altered linolenic acid concentration, based on germplasm from the population N93-194 \times N85-2176. Selections represented all possible homozygous classes of segregates for *Fan* and *Fan* alleles.

isoflavone content in soybean may range from 300 $\mu\text{g/g}$ to greater than 3,000 $\mu\text{g/g}$ among accessions of the USDA soybean germplasm collection (24). Although little is known about the genetic regulation of isoflavone synthesis in soybean, several genes in the phenylpropanoid synthetic pathway have been isolated and cloned (216). Isoflavone synthase (IFS) catalyzes the first committed step of the isoflavone branch of this pathway. IFS is a type of cytochrome P450 protein for which two genes have been identified in soybean. Understanding the genetic regulation of this pathway may become necessary because of interest to maintain adequate isoflavone levels in response to certain genetic and environmental influences. For example, total isoflavone content of soybean seed appears to be negatively related to growth temperature (217). In addition, a negative correlation may exist between total isoflavone content and C18:3 concentration. More recently, data suggests a negative correlation between isoflavone content and higher protein concentration (Fig. 14.6). Therefore, control of isoflavone content may become an important consideration in the development of high-protein soyfoods cultivars.

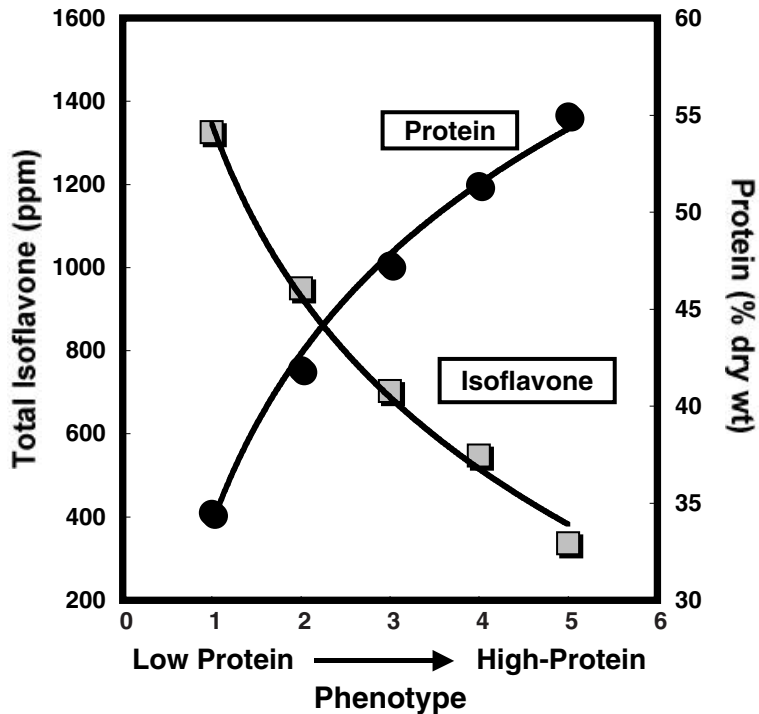


Figure 14.6. Relation of total isoflavone and protein concentration among soybean cultivars.

Summary

In this chapter, the authors have reviewed and discussed the history of genetic enhancement of soybean for soyfoods applications. Future innovations in this technology will involve fundamental changes in the constituent composition of soybean seed. Much of the technology required to attempt this task is already available. However, the simultaneous melding of all the genes that mediate desired changes in protein, oil, and carbohydrate in an agronomic background will necessitate a long-term process for pyramiding these traits in a stepwise and orderly manner. Ultimately, soyfoods varieties will have seeds with higher protein and oil, improved amino acid balance, increased sugar content, and increased protein functionality. The soybean meal used for new soyfoods products then will have stable isoflavone content, and possibly reduced oligosaccharides (and increased soluble sugars). Notwithstanding important alterations in seed composition, the foremost feature of these cultivars must be very competitive yielding ability. This goal is attainable and will be achieved. Together, these innovations should stimulate market demand for soyfoods products.

Acknowledgments

We thank Dr. Keisuke Kitamura for his valuable suggestions, and the former Japanese soybean breeders Drs. Isao Matsukawa, Shigeki Nakamura, Nobuo Takahashi, and Kazunori Igita for preparing [Table 14.7](#).

References

1. Gai, J., Soybean Breeding, in *Plant Breeding: Crop Species* [In Chinese], edited by J. Gai, China Agriculture Press, Beijing, China, 1997, p. 207–251.
2. Gai, J., and W. Guo, History of Maodou Production in China, in *Proceedings of the Second International Vegetable Soybean Conference (Edamame/Maodou)*, Tacoma, Washington, August 10–11, 2001, edited by T.A. Lumpkin and S. Shanmugasundaram, Washington State University, Pullman, 2001, pp. 41–47.
3. Qiu, L., R. Chang, J. Sun, X. Li, Z. Cui, and Z. Li, The History and Use of Primitive Varieties in Chinese Soybean Breeding, in *Proceedings of the World Soybean Research Conference VI, Chicago, IL, 4-7 Aug. 1999*, edited by H.E. Kauffman, AOCS Press, Champaign, Illinois, 1999, pp. 165–172.
4. Hymowitz, T., and J.R. Harlan, Introduction of Soybean to North America by Samuel Bowen in 1765, *Econ. Bot.* 37:371–379 (1983).
5. Chang, R., L. Qiu, J. Sun, Y. Chen, X. Li, and Z. Xu, Collection and Conservation of Soybean Germplasm in China, in *Proceedings of the World Soybean Research Conference VI, Chicago, IL, 4-7 Aug. 1999*, edited by H.E. Kauffman, AOCS Press, Champaign, Illinois, 1999, pp. 172–176.
6. Carter, T.E., Jr., R.L. Nelson, C. Sneller, and Z. Cui, Genetic Diversity in Soybean, in *Soybean Monograph*, 3rd ed., edited by H.R. Boerma and J.E. Specht, American Society of Agronomy, Madison, Wisconsin, 2004, pp. 303–415.
7. Yu, C.L., and A. Buckwell, *Chinese Grain Economy and Policy*, CAB International, London, 1991.

8. Lu, M, and L. Wang, State of the soybean industries in the People's Republic of China, in *Proceedings of the World Soybean Research Conference VI, Chicago, IL, 4-7 Aug. 1999*, edited by H.E. Kauffman, AOCS Press, Champaign, Illinois, 1999, pp. 1–5.
9. Chang, R.Z., J.Y. Sun, and L.J. Qiu, Evolution and Development of Soybean Varieties and Research Plans of Soybean Germplasm [In Chinese], *Soybean Bull.* 2(3):35–36 (1993).
10. Cui, Z., J. Gai, T.E. Carter, Jr., J. Qiu, and T. Zhao, The Released Soybean Cultivars and Their Pedigree Analyses (1923–1995) [In Chinese], China Agriculture Press, Beijing, China, 1998.
11. Cui, Z., T.E. Carter, Jr., J. Gai, J. Qiu, and R.L. Nelson, Origin, Description, and Pedigree of Chinese Soybean Cultivars Released from 1923 to 1995, U.S. Department of Agriculture Technical Bulletin 1871, U.S. Government Printing Office, Washington, DC, 1999.
12. Jin, J., and J. Gai, A Study on Genetic Variation of Tofu Yield, Quality and Processing Traits of Soybean Landraces [In Chinese, with English abstract], *J. Nanjing Agric. Univ.* 18:5–9 (1995).
13. Qian, H., J. Gai, D. Ji, and M. Wang, Correlations of Tofu Yield and Quality with Seed Nutrients and Processing Traits [In Chinese, with English abstract], *J. Chinese Cereals Oils Assoc.* 14(5):35–39 (1999).
14. Qian, H., D. Yu, M. Wang, Q. Song, and J. Gai, Genetic Variation among Landraces and Inheritance of Soymilk and Tofu Processing-Related Traits, in *Proceedings of the World Soybean Research Conference VI, Chicago, IL, 4-7 Aug. 1999*, edited by H.E. Kauffman, AOCS Press, Champaign, Illinois, 1999, pp. 481–482.
15. Gai, J., and H. Qian, A Study on the Inheritance of Dried Tofu Output of Soybeans, in *The Japanese Society for Food and Technology and the Organizing Committee for ISPUC-III 2000*, pp. 47–48.
16. Guo, W., *The History of Soybean Cultivation in China* [In Chinese], Hehai University Press, Nanjing, Jiangsu, China, 1993.
17. Carter, T.E., Jr., and S. Shanmugasundaram, Edamame, the Vegetable Soybean, in *Underutilized Crops: Pulses and Vegetables*, edited by T. Howard, Chapman and Hill, London, 1993, pp. 219–239.
18. Cui, Z., T.E. Carter, Jr., J.W. Burton, and R. Wells, Phenotypic Diversity of Modern Chinese and North American Soybean Cultivars, *Crop Sci.* 41:1954–1967 (2001).
19. Gai, J., and Z. Cui, Studies on Gene Resources of Soybeans from Southern China for Specific Breeding Purposes, in *Proceedings of WSRC V*, edited by Banpot Napompeth, Kasetsart University Press, Bangkok, Thailand, 1997, pp. 60–63.
20. Xu, Z., R. Chang, L. Qiu, J. Sun, and X. Li, Evaluation of Soybean Germplasm in China, in *Proceedings of the World Soybean Research Conference VI, Chicago, IL, 4-7 Aug. 1999*, edited by H.E. Kauffman, AOCS Press, Champaign, Illinois, 1999, pp. 156–165.
21. Hymowitz, T., Dorsett-Morse Soybean Collection Trip to East Asia: 50 Year Retrospective, *Econ. Bot.* 38:378–388 (1984).
22. Bernard, R.L., G.A. Juvik, E.E. Hartwig, and C.J. Edwards, Jr., Origins and Pedigrees of Public Soybean Varieties in the United States and Canada, U.S. Department of Agriculture Technical Bulletin 1746, U.S. Gov. Print. Office, Washington, DC, 1988.
23. Simonne, A.H., D.B. Weaver, and C.I. Wei, Immature Soybean Seeds as a Vegetable or Snackfood: Acceptability by American Consumers, *Innov. Food Sci. Emerging Technol.* 1:289–296 (2001).

24. U.S. Department of Agriculture, Agricultural Research Service, National Genetic Resources Program, Germplasm Resources Information Network (GRIN) [Online Database], National Germplasm Resources Laboratory, Beltsville, Maryland. Available at www.ars-grin.gov/var/apache/cgi-bin/npgs/html/ (accessed October 1, 2001).
25. Iowa State University Website. Available at www.ag.iastate.edu/centers/cad/specialtysoyt.html (accessed July 7, 2004).
26. Malecot, G., *The Mathematics of Heredity*, W.H. Freeman and Co., San Francisco, California, 1969. Originally published as *Les Mathematiques de l'Heredité* (Masson, Paris, 1948).
27. Cui, Z., Carter, T.E., Jr., and Burton, J.W., Genetic Diversity Patterns of Chinese Soybean Cultivars Based on Coefficient of Parentage, *Crop Sci.* 40:1780–1793 (2000).
28. Kihara, H., History of Biology and Other Sciences in Japan in Retrospect, in *Proceedings of the XII International Congress of Genetics* [In Japanese], edited by C. Oshima, The Science Council of Japan, 1969, p. 49–70.
29. Sugiyama, S., On the Origin of Soybean, *Glycine max* Merrill [In Japanese], *J. Brewing Soc. Jap.* 87:890–899 (1992).
30. Li, Z., and R.L. Nelson, Genetic Diversity among Soybean Accessions from Three Countries Measured by RAPDs, *Crop Sci.* 41:1337–1347 (2001).
31. Carter, T.E., Jr., R.L. Nelson, P.B. Cregan, H.R. Boerma, P. Manjarrez-Sandoval, X. Zhou, W.J. Kenworthy, and G.N. Ude, Project SAVE (Soybean Asian Variety Evaluation)—Potential New Sources of Yield Genes with No Strings from USB, Public, and Private Cooperative Research, in *Proceedings of the 28th Soybean Seed Research Conference 1998*, edited by B. Park, American Seed Trade Association, Washington DC, 2000, pp. 68–83.
32. Anonymous, *Soybean Production Yearbook*, Ministry of Agriculture, Forestry and Fisheries, Crop Production Division, Japan, 2001, p. 282.
33. Saito, M., Breeding of Soybean in Japan, in *Proceedings of a Symposium on Tropical Agriculture Research 12–14 September, 1972*, Tropical Agric. Res. Series No. 6, Tropical Agriculture Research Center, Tskuba, Japan, 1972, pp. 43–54.
34. Kaizuma, N., and J. Fukui, Breeding Soybean for Chemical Quality in Japan, in *Proceedings of a Symposium on Tropical Agriculture Research 12–14 September, 1972*, Tropical Agric. Res. Series No. 6, Tropical Agriculture Research Center, Japan, 1972, pp. 55–68.
35. Miyazaki, S., T.E. Carter, Jr., S. Hattori, H. Nemoto, T. Shina, E. Yamaguchi, S. Miyashita, and Y. Kunihiro, Identification of Representative Accessions of Japanese Soybean Varieties Registered by Ministry of Agriculture, Forestry and Fisheries, Based on Passport Data Analysis, No. 8, Misc. Pub. of the National Institute of Agrobiological Resources (Japan), 1995.
36. Zhou, X., T.E. Carter, Jr., Z. Cui, S. Miyazaki, and J.W. Burton, Genetic Diversity Patterns in Japanese Soybean Cultivars Based on Coefficient of Parentage, *Crop Sci.* 42:1331–1342 (2002).
37. Zhou, X., T.E. Carter, Jr., Z. Cui, S. Miyazaki, and J.W. Burton, Genetic Base of Japanese Soybean Cultivars Released during 1950 to 1988, *Crop Sci.* 40:1794–1802 (2000).
38. Taira, H., Quality of Soybeans for Processed Foods in Japan, *Jap. Agric. Res. Q.* 24:224–230 (1990).

39. Kitamura, K., Spontaneous and Induced Mutations of Seed Proteins in Soybean (*Glycine max* L. Merrill), Gamma Field Symposia No.30, (Shoji—Need publisher and editor), 1991, pp. 46–54.
40. Kitamura, K., Breeding Trials for Improving the Food-Processing Quality of Soybeans, *Trends Food Sci. Technol.* 4:64–67 (1993).
41. Taira, H., Methods of Evaluating Soybean Quality for Natto and Nimame, in *Shokuryo-Food Science and Technology 40* [In Japanese], National Food Research Institute (Japan), 2002, pp. 153–168.
42. Hajika, M., M. Takahashi, S. Sakai, and K. Igita, A New Genotype of 7 S Globulin (β -Conglycinin) Detected in Wild Soybean (*Glycine soja* Sieb. et Zucc.), *Breed. Sci.* 46:385–386 (1996).
43. Takahashi, K., H. Banba, A. Kikuchi, M. Ito, and S. Nakamura, An Induced Mutant Line Lacking the α -Subunit of β -Conglycinin in Soybean (*Glycine max* (L.) Merrill), *Breed. Sci.* 44:65–66 (1994).
44. Takahashi, K., Y. Mizuno, S. Yumoto, K. Kitamura, and S. Nakamura, Inheritance of the α -Subunit Deficiency of β -Conglycinin in Soybean (*Glycine max* (L.) Merrill) Line Induced by γ -Ray Irradiation, *Breed. Sci.* 46:251–255 (1996).
45. Gray, S.G., Experiments with Soybeans in Australia, Division of Plant Industry Technical Paper No. 4, Commonwealth Scientific and Industrial Research Organisation, Melbourne, 1955.
46. James, A.T., Varietal Evaluation of Soybean for Culinary Quality, in *Soybean 2000, Advancing Soybean into the New Millenium. Proceedings: of the 11th Australian Soybean Conference, Ballina, Australia*, edited by P. Desborough, NSW Agriculture, 2000, pp. 45–48.
47. Wilson, L.A., Soy Foods, in *Practical Handbook of Soybean Processing and Utilization*, edited by D.R. Erickson, AOCS Press, Champaign, Illinois, and United Soybean Board, St Louis, Missouri, 1995, pp. 428–459.
48. Johnson, L.D., and L.A. Wilson, Influence of Soybean Variety and Method of Processing in Tofu Manufacturing: Comparison of Methods for Measuring Soluble Solids in Soymilk, *J. Food Sci.* 49:202–204 (1984).
49. Nakamura, T.S., T.S. Utsumi, K., Kitamura, K. Harada, and T. Mori, Cultivar Differences in Gelling Characteristics of Soybean Glycinin, *J. Agric. Food Chem.* 32:647–651 (1984).
50. Lim, B.T., J.M. de Man, L. de Man, and R.I. Buzzell, Yield and Quality of Tofu as Affected by Soybean and Soymilk Characteristics: Calcium Sulfate Coagulant, *J. Food Sci.* 55:1088–1092 (1990).
51. Evans, B.E., C. Tsukamoto, and N.C. Neilsen, A Small Scale Method for the Production of Soymilk and Silken Tofu, *Crop Sci.* 37:1463–1471 (1997).
52. Cai, T., and K. Chang, Processing Effect on Soybean Storage Proteins and Their Relationship with Tofu Quality, *J. Agric. Food Chem.* 47:720–727 (1999).
53. James, A.T., and E.E. Bumstead, Genotypic Variation in Australian Soybean Cultivars for Tofu Quality Traits, in *Plant Breeding for the 11th Millenium, Proceedings of the 12th Australian Plant Breeding Conference, Perth, W. Australia*, edited by J.A. McComb, Australian Plant Breeding Assoc., Inc, 2002, pp. 770–773.
54. Kijima, H., Manufacture of Tofu, in *Science of Tofu*, edited by T. Watanabe, Food Journal Co., Ltd., Kyoto, Japan, 1997, pp. 14–29.
55. Hou, H.J., and S.K.C. Chang, Yield and Quality of Soft Tofu as Affected by Soybean Physical Damage and Storage, *J. Agric. Food Chem.* 46:4798–4805 (1998).

56. Saio, K., What Are Soybeans?, in *Science of Tofu*, edited by T. Watanabe, Food Journal Co., Ltd., Kyoto, Japan, 1997, pp. 77–103.
57. Sasaki, I., Material Soybeans, in *Science of Tofu*, edited by T. Watanabe, Food Journal Co., Ltd. Kyoto, Japan, 1997, pp. 68–76.
58. Gai, J., H. Qian, D. Ji, and M. Wang, A Study on Inheritance of Dried Tofu Output of Soybean, *Acta Genetica Sinica* 27:434–439 (2000).
59. Jin, J., and J. Gai, Correlation Analysis Regarding Tofu Yield, Quality and Processing Traits of Soybean Landraces. [In Chinese, with English abstract], *Scientia Agricultura Sinica* 29:28–33 (1996).
60. Bhardwaj, H.L., A.S. Bhagsari, J.M. Joshi, M. Rangappa, V.T. Sapra, and M.S.S. Rao, Yield and Quality of Soymilk and Tofu Made from Soybean Genotypes Grown at Four Locations, *Crop Sci.* 39:401–405 (1999).
61. Hartwig, E.E., and C.J. Edwards, Effect of Morphological Characteristics on Seed Yield of Soybeans, *Agron. J.* 62:64–65 (1970).
62. Mies, D.W., and T. Hymowitz, Comparative Electrophoretic Studies of Trypsin Inhibitors in Seed of the Genus *Glycine*, *Bot. Gaz.* 134:121–125 (1973).
63. Mityko, J., J. Batkai, and G. Hodos-Kotvics, Trypsin Inhibitor Content in Different Varieties and Mutants of Soybean, *Acta Agron. Hungarica* 39:401–405 (1990).
64. Werner, M.H., and D.E. Wemmer, H Assignments and Secondary Structure Determination of the Soybean Trypsin/Chymotrypsin Bowman-Birk Inhibitor, *Biochemistry* 30:3356–3364 (1991).
65. Coates, J.B., J.S. Medeiros, V.H. Thanh, and N.C. Nielsen, Characterization of the Subunits of β -Conglycinin, *Arch. Biochem. Biophys.* 243:184–194 (1985).
66. Maruyama, N., M. Adachi, K. Takahashi, K. Yagasaki, M. Kohno, Y. Takenaka, E. Okuda, S. Nakagawa, B. Mikami, and S. Utsumi, Crystal Structures of Recombinant and Native Soybean β -Conglycinin β Homotrimers, *Eur. J. Biochem.* 268:3595–3604 (2001).
67. Miles, M.J., V.J. Morris, D.J. Wright, and J.R. Bacon, A Study of the Quaternary Structure of Glycinin, *Biochim. Biophys. Acta* 827:119–126 (1985).
68. Nielsen, N.C., The Structure and Complexity of the 11S Polypeptides in Soybeans, *J. Am. Oil Chem. Soc.* 62:1680–1685 (1985).
69. Nielsen, N.C., V. Beilinson, R. Bassuner, and S. Reverdatto, A Gb-like Protein from Soybean, *Physiol. Plant.* 111:75–82 (2001).
70. Nielsen, N.C., R. Jung, Y.-W. Nam, T.W. Beaman, L.O. Oliveira, and R. Bassuner, Synthesis and Assembly of 11S Globulins, *J. Plant Physiol.* 145:641–647 (1995).
71. Nielsen, N.C., C.D. Dickinson, T. J. Cho, V.H. Thanh, B.J. Scallon, R.L. Fischer, T.L. Sims, G.N. Drews, and R.B. Goldberg, Characterization of the Glycinin Gene Family in Soybean, *Plant Cell* 1:313–328 (1989).
72. Watanabe, Y., and H. Hirano, Nucleotide Sequence of the Basic 7S Globulin Gene from Soybean, *Plant Physiol.* 105:1019–1020 (1994).
73. Saio, K., and T. Watanabe, Differences in Functional Properties of 7S and 11S Soybean Proteins, *J. Texture Studies* 9:135–157 (1978).
74. Saio, K., M. Kamiya, and T. Watanabe, Effect of Differences of Protein Components among Soybean Varieties on Formation of Tofu-Gel, *Agric. Biol. Chem.* 33:1301–1308 (1969).
75. Mori, T., S. Utsumi, H. Inaba, K. Kitamura, and K. Harada, Differences in Subunit Composition of Glycinin among Soybean Cultivars, *J. Agric. Food Chem.* 29:20–23 (1981).

76. Hughes, S.A., and P.A. Murphy, Varietal Influence on the Quantity of Glycinin in Soybeans, *J. Agric. Food Chem.* 31:376–379 (1983).
77. Wolf, W.J., G.E. Babcock, and A.K. Smith, Ultracentrifugal Differences in Soybean Protein Composition, *Nature* 191:1395–1396 (1961).
78. Wolf, W.J., G.E. Babcock, and A.K. Smith, Purification and Stability Studies of 11-S Component of Soybean Proteins, *Arch. Biochem. Biophys.* 99:265–274 (1962).
79. Kitamura, K., Genetic Improvement of Nutritional and Food Processing Quality in Soybean, *Jap. Agric. Res. Q.* 29:1–8 (1995).
80. Ji, M.P., T.D. Cai, and K.C. Chang, Tofu Yield and Textural Properties from Three Soybean Cultivars as Affected by Ratios of 7S and 11S Proteins, *J. Food Sci.* 64:763–767 (1999).
81. Xu, B., S.H. Zou, B.C. Zhuang, Z.P. Lin, and Y.J. Zhao, Study on Seed Storage Component 11S/7S of Wild Soybean (*G. soja*) [In Chinese, with English abstract], *Acta Agronomica Sinica* 16:235–241 (1990).
82. Harada, K., and K.G. Hossain, Genetic Variation of Protein Composition in Soybean Seeds, in *Japan Part of Proceedings of the International Conference on Soybean Processing and Utilization, China*, June 25–29, 1990, edited by K. Okubo, K. Kijima, S. Saio, T. Inoue, and T. Watanabe, Publishing by Japanese Committee, 1991.
83. Kim, Y.H., S.D. Kim, and E.H. Hong, 11S and 7S Globulin Fractions in Soybean Seed and Soycurd Characteristics, *Korean J. Crop Sci.* 39:348–352 (1995).
84. Yagasaki, K., F. Kousaka, and K. Kitamura, Potential Improvement of Soymilk Gelation Properties by Using Soybeans with Modified Protein Subunit Compositions, *Breed. Sci.* 50:101–107 (2000).
85. Tezuka, M., H. Taira, Y. Igarashi, K. Yagasaki, and T. Ono, Properties of Tofus and Soymilks Prepared from Soybeans Having Different Subunits of Glycinin, *J. Agric. Food Chem.* 48:1111–1117 (2000).
86. Murphy, P.A., and A.P. Resurreccion, Varietal and Environmental Differential Differences in Soybean Glycinin and b-Conglycinin, *J. Agric. Food Chem.* 32:911–915 (1984).
87. Srinivasan, A., and J. Arihara, Soybean Seed Discoloration and Cracking in Response to Low Temperatures during Early Reproductive Growth, *Crop Sci.* 34:1611–1617 (1994).
88. Takahashi, R., Association of Soybean Genes I and T with Low-Temperature Induced Seed Coat Deterioration, *Crop Sci.* 37:1755–1759 (1997).
89. Morrison, M.J., L.N. Pietrzak, and H.D. Voldeng, Soybean Seed Coat Discoloration in Cool-Season Climates, *Agron. J.* 90:471–474 (1998).
90. Takahashi, R., and J. Abe, Soybean Maturity Genes Associated with Seed Coat Pigmentation and Cracking in Response to Low Temperatures, *Crop Sci.* 39:1657–1662 (1999).
91. Wilson, R.F., Seed Metabolism, in *Soybeans: Improvement, Production and Uses.*, edited by J.R. Wilcox, American Society of Agronomy, Madison, Wisconsin, 1987, pp. 643–686.
92. Krober, O.A., and J.L. Cartter, Quantitative Interrelations of Protein and Nonprotein Constituents of Soybeans, *Crop Sci.* 2:171–172 (1962).
93. Geater, C.W., W.R. Fehr, and L.A. Wilson, Association of Soybean Seed Traits with Physical Properties of Natto, *Crop Sci.* 40:1529–1534 (2000).
94. Hartwig, E.E., T.M. Kuo, and M.M. Kenty, Seed Protein and Its Relationship to Soluble Sugars in Soybean, *Crop Sci.* 37:770–773 (1997).
95. Wilcox, J.R., and R.M. Shibles, Interrelationships among Seed Quality Attributes in Soybean, *Crop Sci.* 41:11–14 (2001).

96. Carrao-Panizzi, M.C., and K. Kitamura, Isoflavone Contents in Brazilian Soybean Cultivars, *Breed. Sci.* 45:295-300 (1994).
97. Carrao-Panizzi, M.C., A.D. Pino Beleia, K. Kitamura, and M.C. Neves Oliveira, Effects of Genetics and Environment on Isoflavone Content of Soybean from Different Regions of Brazil, *Brasilia* 34:1787-1795 (1999).
98. Mandarino, J.M.G., M.C. Carrao-Panizzi, and M. Shiraiwa, Composition and Content of Saponins in Soybean Seeds of Brazilian Cultivars and Breeding Lines, in *Proceedings of the Third International Soybean Processing and Utilization Conference, Oct. 15-20, 2000, Tsukuba, Japan*, edited by K. Saio, Korin Publishing Co., Ltd., Japan, 2000, pp. 61-62.
99. Takemura, H., N. Ando, and Y. Tsukamoto, Breeding of Branched Short-Chain Fatty Acids Non-producing Natto Bacteria and Its Application to Production of Natto with Light Smells, *Nippon Shokuhin Kagaku Kogaku Kaishi* 47:773-779 (2000).
100. Muramatus, K., T. Katsumata, S. Watanabe, T. Tanaka, and K. Kiuchi, Development of Low-Flavour Natto Manufactured with Leucine-Requiring Mutants of Elastase-Producing *Bacillus natto*, *Nippon Shokuhin Kagaku Kogaku Kaishi* 48:287-298 (2001).
101. Carter, T.E., Jr., and R.F. Wilson, Soybean Quality for Human Consumption, *Proc. Australian Conf.* 10:1-16 (1998).
102. Carter, T.E., Jr., Genetic Alteration of Soybean Seed Size: Breeding Strategies and Market Potential, *Proc. Am. Seed Trade Assoc.* 17:33-45 (1988).
103. Brar, G.S., and T.E. Carter, Jr., Soybean, *Glycine max* (L.) Merrill, in *Genetic Improvement of Vegetable Crops*, edited by G. Kalloo and B.O. Bergh, Pergamon Press, Oxford, 1993, pp. 427-463.
104. Hsu, K.H. C.J. Kim, and L.A. Wilson, Factors Affecting Water Uptake of Soybean during Soaking, *Cereal Chem.* 60:208-211 (1983).
105. Mwandemele, O.D., K.S. McWhirter, and C. Chesterman, Genetic Variation in Soybean (*Glycine max* (L.) Merril) for Cooking Ability and Water Absorption during Cooking, *Euphytica* 33:859-864 (1984).
106. Mwandemele, O.D., K.S. McWhirter, and C. Chesterman, Improving the Quality of Soybean (*Glycine max* (L.) Merr.) for Human Consumption: Factors Influencing the Cookability of Soybean Seeds, *J. Food Sci. Technol.* 21:286-290 (1984).
107. Mwandemele, O.D., and A. Doto, Evaluation of Soybean Lines for Drought Tolerance and the Influence of Water Availability on Cookability, *Turrialba*. 38:194-197 (1988).
108. Nielson, S.S., W.E. Brandt, and B.B. Singh, Genetic Variability for Nutritional Composition and Cooking Time of Improved Cowpea Lines, *Crop Sci.* 33:469-472 (1993).
109. Wei, Q., C. Wolf-Hall, and K.C. Chang, Natto Characteristics as Affected by Steaming Time, Bacillus Strain, and Fermentation Time, *J. Food Sci.* 66:167-173 (2001).
110. Kanno, A., H. Takamatsu, N. Takano, and T. Akimoto, Change of Saccharides in Soybeans during Manufacturing of Natto, *Nippon Shokuhin Kogyo Gakkaishi* 29:105-110 (1982).
111. Keiser, J.R., and R.E. Mullen, Calcium and Relative Humidity Effects on Soybean Seed Nutrition and Seed Quality, *Crop Sci.* 33:1345-1349 (1993).
112. Raboy, V., D.B. Dickinson, and F.E. Below, Variation in Seed Total Phosphorous, Phytic Acid, Zinc, Calcium, Magnesium, and Protein among Lines of *Glycine max* and *G. soja*, *Crop Sci.* 24:431-434 (1984).
113. Laszlo, J.A., Mineral Contents of Soybean Seed Coats and Embryos during Development, *J. Plant Nutr.* 13:231-248 (1990).

114. Burton, M.G., M.J. Laurer, and M.B. McDonald, Calcium Effects on Soybean Seed Production, Elemental Concentration, and Seed Quality, *Crop Sci.* 40:476–482 (2000).
115. Cober, E.R., J.A. Fregeau-Read, L.N. Pietrzak, A.R. McElroy, and H.D. Voldeng, Genotype and Environmental Effects on Natto Soybean Quality Traits, *Crop Sci.* 37:1151–1154 (1997).
116. Yinbo, G., M.B. Peoples, and B. Rerkasem, The Effect of N Fertilizer Strategy on N₂ Fixation, Growth and Yield of Vegetable Soybean, *Field Crop Res.* 51:221–229 (1997).
117. Fehr, W.R., C.E. Caviness, D.T. Burwood, and J.S. Pennington, Stage of Development Description of Soybean (*Glycine max* (L) Merr.), *Crop Sci.* 11:929–930 (1971).
118. Mebrahtu, T., A. Mohamed, and W. Mersie, Green Pod and Architectural Traits of Selected Vegetable Soybean Genotypes, *J. Prod. Agric.* 4:395–399 (1991).
119. Rao, M.S.S., B.G. Mullinix, M. Rangappa, E. Cebert, A.S. Bhagsari, V.T. Sapra, J. M. Joshi, and R.B. Dadson, Genotype × Environment Interactions and Yield Stability of Food Grade Soybean Genotypes, *Agron. J.* 94:72–80 (2002).
120. Shanmugasundaram, S., S.C.S. Tsou, and T.L Hong, Vegetable Soybeans Production and Research, in *Proceedings of World Soybean Research Conference V, Chiang Mai, Thailand. 21–27 Feb. 1994*, edited by B. Napompeth, Kasetsart University Press, Bangkok, 1997, pp. 529–532.
121. Konovsky, J., T.A. Lumpkin, and D. McClary, Edamame: The Vegetable Soybean, in *Understanding the Japanese Food and Agrimarket: A Multifaceted Opportunity*, edited by A.D. O'Rourke, Hayworth, Binghamton, U.K., 1994, pp. 173–181.
122. Hikino, I., Super Premium Variety “Tanbaguro,” in *Proceedings of the Third International Soybean Processing and Utilization Conference 2000: Dawn of the Innovative Era for Soybeans*, Korin Publishing Co., Ltd., Tsukuba, Japan, 2000, pp. 35–36.
123. Masuda, R., and K. Harada, Carbohydrate Accumulation in Developing Soybean Seeds; Sucrose and Starch Levels in 30 Cultivars for Soyfoods, in *Proceedings of the Third International Soybean Processing and Utilization Conference 2000: Dawn of the Innovative Era for Soybeans*, Korin Publishing Co. Ltd, Tsukuba, Japan, 2000, pp. 67–68.
124. Young, G., T. Mebrahtu, and J. Johnson, Acceptability of Green Soybeans as a Vegetable Entity, *Plant Foods Hum. Nutr.* 55:323–333 (2000).
125. Chotiarnwong, A., P. Chotiarnwong, W. Gong-in, A. Nalampang, N. Potan, V. Benjasil, and V. Kajornmalle, Chiang Mai 1—A Vegetable Soybean Released in Thailand, *Tropical Vegetable Information Service Newsletter* 1:12 (1996).
126. Kanthamani, S., A.I. Nelson, and M.P. Steinburg, Home Preparation of Soymilk: A New Concept, in *Whole-Soybean Foods for Home and Village Use*, edited by A.I. Nelson *et al.*, International Agricultural Publications, INTSOY Series No. 14, University of Illinois, 1978, pp. 5–11.
127. Chen, S., Principles of Soymilk Production, in *Food Uses of Whole Oil and Protein Seeds*, edited by E.W. Lucas, *et al.*, AOCS Press, Champaign, Illinois, 1989, pp. 40–86.
128. Mullin, W.J., J.A. Fregeau-Reid, M. Butler, V. Poysa, L. Woodrow, D.B. Jessop, and D. Raymond, An Interlaboratory Test of a Procedure to Assess Soybean Quality for Soymilk and Tofu Production, *Food Res. Int.* 34:669–677 (2001).
129. Satterfield, M., D.M. Black, and J.S. Brodbelt, Detection of the Isoflavone Aglycones Genistein and Diadzein in Urine Using Solid-Phase Microextraction-High-Performance

Liquid Chromatography-Electrospray Ionisation Mass Spectrometry, *J. Chromatogr. B* 759:3341–3346 (2001).

130. Jackson, C.J.C., J.P. Dini, C. Lavandier, H.P.V. Rupasinghe, H. Faulkner, V. Poysa, D. Buzzell, and S. De Grandis, Effects of Processing on the Content and Composition of Isoflavones during Manufacturing of Soy Beverage and Tofu, *Process Biochem.* 37:1117–1123 (2002).
131. Budziszewski, G.J., K.P.C. Croft, and D.F. Hildebrand, Uses of Biotechnology in Modifying Plant Lipids, *Lipids* 31:557–569 (1996).
132. Kinney, A.J., Improving Soybean Seed Quality, *Curr. Opin. Biotechnol.* 5:144–151 (1994).
133. Hurburgh, C.R., Jr., T.J. Brumm, J.M. Guinn, and R.A. Hartwig, Protein and Oil Patterns in U.S. and World Soybean Markets, *J. Am. Oil Chem. Soc.* 67:966–973 (1990).
134. Wilcox, J.R., and Z. Guodong, Relationships between Seed Yield and Seed Protein in Determinate and Indeterminate Soybean Populations, *Crop Sci.* 37:361–364 (1997).
135. Kenworthy, W.J., and C.A. Brim, Recurrent Selection in Soybeans. I. Seed Yield, *Crop Sci.* 19:315–318 (1979).
136. Wilcox, J.R., Increasing Seed Protein in Soybean with Eight Cycles of Recurrent Selection, *Crop Sci.* 38:1536–1540 (1998).
137. Burton, J.W., T.E. Carter, Jr., and R.F. Wilson, Registration of ‘Prolina’ Soybean, *Crop Sci.* 39:294–295 (1999).
138. Altenbach, S.B., C.-C. Kuo, L.C. Staraci, K.W. Pearson, C. Wainwright, A. Georgescu, and J. Townshend, Accumulation of a Brazil Nut Albumin in Seeds of Transgenic Canola Results in Enhanced Levels of Seed Protein Methionine, *Plant Mol. Biol.* 18:235–245 (1992).
139. Ampe, C., J. Van Damme, L.A.B. Castro, M.J.A.M. Sampaio, M. Van Montagu, and J. Vandekerckhove, The Amino-Acid Sequence of the 2S Sulphur-Rich Proteins from Seed of Brazil Nut (*Bertholletia excelsa* H.B.K.), *Eur. J. Biochem.* 159:597–604 (1986).
140. Sun, S.S.M., F.W. Leung, and J.C. Tomic, Brazil Nut (*Bertholletia excelsa* H.B.K.) Proteins: Fractionation, Composition, and Identification of a Sulphur-Rich Protein, *J. Agric. Food Chem.* 35:232–235 (1987).
141. Mandal, S., and R.K. Mandal, Seed Storage Proteins and Approaches for Improvement of Their Nutritional Quality by Genetic Engineering, *Curr. Sci.* 79:576–589 (2000).
142. Harada, J.J., S.J. Barker, and R.B. Goldberg, Soybean β -Conglycinin Genes Are Clustered in Several DNA Regions and Are Regulated by Transcriptional and Posttranscriptional Processes, *Plant Cell* 1:415–425 (1989).
143. Lessard, P.A., R.D. Allen, T. Fujiwara, and R.N. Beachy, Upstream Regulatory Sequences from Two β -Conglycinin Genes, *Plant Mol. Biol.* 22:873–885 (1993).
144. Tierney, M.L., E.A. Bray, R.D. Allen, Y. Ma, R.F. Drong, J. Slightom, and R.N. Beachy, Isolation and Characterization of a Genomic Clone Encoding the β -Subunit of β -Conglycinin, *Planta* 172:356–363 (1987).
145. Beachy, R.N., J. Bryant, J.J. Doyle, K. Kitamura, and B.F. Ladin, Molecular Characterization of a Soybean Variety Lacking a Subunit of the 7S Seed Storage Protein, *Plant Mol. Biol.* 23:413–422 (1983).
146. Yoshino, M., A. Kanazawa, K.-I. Tsutsumi, I. Nakamura, and Y. Shimamoto, Structure and Characterization of the Gene Encoding a Subunit of Soybean β -Conglycinin, *Genes Genet. Syst.* 76:99–105 (2001).

147. Kinney, A.J., R. Jung, and E.M. Herman, Cosuppression of the α subunits of β -Conglycinin in Transgenic Soybean Seeds Induces the Formation of Endoplasmic Reticulum-Derived Protein Bodies, *Plant Cell* 13:1165–1178 (2001).
148. Phan, T.H., N. Kaizuma, H. Odanaka, and Y. Takahata, Specific Inheritance of a Mutant Gene Controlling α , β Subunits-Null of β -Conglycinin in Soybean (*Glycine max* (L.) Merr.) and Observation of Chloroplast Ultrastructure of the Mutant, *Breed. Sci.* 46:53–59 (1996).
149. Hayashi, M., M. Nishioka, K. Kitamura, and K. Harada, Identification of AFLP Markers Tightly Linked to the Gene for Deficiency of the 7S Globulin in Soybean Seed and Characterization of Abnormal Phenotypes Involved in the Mutation, *Breed. Sci.* 50:123–129 (2000).
150. Ogawa, T., E. Tayama, K. Kitamura, and N. Kaizuma, Genetic Improvement of Seed Storage Proteins Using Three Variant Alleles of 7S Globulin Subunits in Soybean (*Glycine max* L.), *Jap. J. Breed.* 39:137–147 (1989).
151. Kwanyuen, P., R.F. Wilson, and J.W. Burton, Soybean Protein Quality, in *Oilseed and Edible Oils Processing Vol. 1*, edited by S.S. Koseoglu, K.C. Rhee, and R.F. Wilson, AOCS Press, Champaign, Illinois, 1998, pp. 284–289.
152. Luck, P.J., T.C. Lanier, C.R. Daubert, R.F. Wilson, and P. Kwanyuen, Functionality and Viscoelastic Behavior of Prolina Soybean Isolate, in *Oilseed Processing and Utilization*, edited by R.F. Wilson, AOCS Press, Champaign, Illinois, 2001, pp. 197–202.
153. Cho, T.-J., C.S. Davies, and N.C. Nielsen, Inheritance and Organization of Glycinin Genes in Soybean, *Plant Cell* 1:329–337 (1989).
154. Yagasaki, K., N. Kaizuma, and K. Kitamura, Inheritance of Glycinin Subunits and Characterization of Glycinin Molecules Lacking the Subunits in Soybean (*Glycine max* (L.) Merr.), *Breed. Sci.* 46:11–15 (1996).
155. Staswick, P.E., and N.C. Nielsen, Characterization of a Soybean Cultivar Lacking Certain Glycinin Subunits, *Arch. Biochem. Biophys.* 223:1–8 (1983).
156. Sims, T.L., and R.B. Goldberg, The Glycinin Gy1 Gene from Soybean, *Nucleic Acids Res.* 17:4386 (1989).
157. Kitamura, Y., M. Arahira, Y. Itoh, and C. Fukazawa, The Complete Nucleotide Sequence of Soybean Glycinin A2B1a Gene Spanning to Another Glycinin Gene A1aB1b, *Nucleic Acids Res.* 18:4245–4246 (1990).
158. Xue, Z.-T., M.-L. Xu, W. Shen, N.-L. Zhuang, W.-M. Hu, and S.C. Shen, Characterization of a *Gy4* Glycinin Gene from Soybean *Glycine max* cv. Forrest, *Plant Mol. Biol.* 18:897–908 (1992).
159. Diers, B.W., V. Beilinson, N.C. Nielsen, and R.C. Shoemaker, Genetic Mapping of the *Gy4* and *Gy5* Glycinin Genes in Soybean and the Analysis of a Variant of *Gy4*, *Theor. Appl. Genet.* 89:297–304 (1994).
160. Chen, Z., and R.C. Shoemaker, Four Genes Affecting Seed Traits in Soybeans Map to Linkage Group F, *J. Hered.* 89:211–215 (1998).
161. Cho, T.-J., C.S. Davies, R.L. Fischer, N.E. Turner, R.B. Goldberg, and N.C. Nielsen, Molecular Characterization of an Aberrant Allele for the *Gy3* Glycinin Gene: A Chromosomal Rearrangement, *Plant Cell* 1:339–350 (1989).
162. Naito, S., M.Y. Hirai, M. Chino, and Y. Komeda, Expression of a Soybean (*Glycine max* (L.) Merr.) Seed Storage Protein Gene in Transgenic *Arabidopsis thaliana* and Its Response to Nutritional Stress and to Absciscic Acid Mutations, *Plant Physiol.* 104:497–503 (1994).

163. Ohtake, N., T. Kawachi, A. Sato, I. Okuyama, H. Fujikake, K. Sueyoshi, and T. Ohyama, Temporary Application of Nitrate to Nitrogen-Deficient Soybean Plants at the Mid- to Late-Stages of Seed Development Increased the Accumulation of the β -Subunit of β -Conglycinin, a Major Seed Storage Protein, *Soil Sci. Plant Nutr.* 47:195–203 (2001).
164. Davies, C.S., J.B. Coates, and N.C. Nielsen, Inheritance and Biochemical Analysis of Four Electrophoretic Variants of β -Conglycinin from Soybean, *Theor. Appl. Genet.* 71:351–358 (1985).
165. Chen, Z.-L., S. Naito, I. Nakamura, and R.N. Beachy, Regulated Expression of Genes Encoding Soybean β -Conglycinins in Transgenic Plants, *Dev. Genet.* 10:112–122 (1989).
166. Sexton, P.J., N.C. Paek, and R. Shibles, Soybean Sulfur and Nitrogen Balance under Varying Levels of Available Sulfur, *Crop Sci.* 38:975–982 (1998).
167. Sexton, P.J., S.L. Naeve, N.C. Paek, and R. Shibles, Sulfur Availability, Cotyledon Nitrogen: Sulfur Ratio, and Relative Abundance of Seed Storage Proteins of Soybean, *Crop Sci.* 38:983–986 (1998).
168. Nakasathien, S., D.W. Israel, R.F. Wilson, and P. Kwanyuen, Regulation of Seed Protein Concentration in Soybean by Supra-optimal Nitrogen Supply, *Crop Sci.* 40:1277–1284 (2000).
169. Morr, C.V., Current Status of Soy Protein Functionality in Food Systems, *J. Am. Oil Chem Soc.* 67:265–271 (1990).
170. Norby, S.W., C.A. Adams, and R.W. Rinne, An Ultrastructural Study of Soybean Seed Development, Department of Agronomy, University of Illinois, Urbana, 1984.
171. Rubel, A., R.W. Rinne, and D.T. Canvin, Protein, Oil and Fatty Acid Composition in Developing Soybean Seeds, *Crop Sci.* 12:739–741 (1972).
172. Yazdi-Samadi, B., R.W. Rinne, and R.D. Seif, Components of Developing Soybean Seeds: Oil, Protein, Sugars, Starch, Organic Acids, and Amino Acids, *Agron. J.* 69:481–486 (1977).
173. Phillips, D.V., D.O. Wilson, and D.E. Dougherty, Soluble Carbohydrates in Legumes and Nodulated Nonlegumes, *J. Agric. Food Chem.* 32:1289–1291 (1984).
174. Obendorf, R.L., M. Horbowicz, A.M. Dickerman, P. Brenac, and M.E. Smith, Soluble Oligosaccharides and Galactosyl Cyclitols in Maturing Soybean Seeds in Planta and in Vitro, *Crop Sci.* 38:78–84 (1998).
175. Handley, L.W., D.M. Pharr, and R.F. McFeeters, Relationship between Galactinol Synthase Activity and Sugar Composition of Leaves and Seeds of Several Crop Species, *J. Am. Soc. Hort. Sci.* 108:600–605 (1983).
176. Hoch, G., T. Peterbauer, and A. Richter, Purification and Characterization of Stachyose Synthase from Lentil (*Lens culinaris*) Seeds: Galactopinitol and Stachyose Synthesis, *Arch. Biochem. Biophys.* 366:75–81 (1999).
177. Bianchi, M.L.P., H.C. Silva, and G.L. Braga, Oligosaccharide Content of Ten Varieties of Dark-Coated Soybeans, *J. Agric. Food Chem.* 32:355–357 (1984).
178. Hymowitz, T., W.M. Walker, F.I. Collins, and J. Panczner, Stability of Sugar Content in Soybean Strains, *Commun. Soil Sci. Plant Anal.* 3:367–373 (1972).
179. Jones, D.A., M.S. DuPont, M.J. Ambrose, J. Frias, and C.L. Hedley, The Discovery of Compositional Variation for the Raffinose Family of Oligosaccharides in Pea Seeds, *Seed Sci. Res.* 9:305–310 (1999).
180. Openshaw, S.J., and H.H. Hadley, Selection to Modify Sugar Content of Soybean Seeds, *Crop Sci.* 21:805–808 (1981).

181. Saravitz, D.M., D.M. Pharr, and T.E. Carter, Jr., Galactinol Synthase Activity and Soluble Sugars in Developing Seeds of Four Soybean Genotypes, *Plant Physiol.* 83:185–189 (1987).
182. Kerr, P.S., and S.A. Sebastian, Soybean Products with Improved Carbohydrate Composition and Soybean Plants, U.S. Patent 5,710,365, January 20, 1998.
183. Hsu, S.H., H.H. Hadley, and T. Hymowitz, Changes in Carbohydrate Contents of Germinating Soybean Seeds, *Crop Sci.* 13:407–410 (1973).
184. Geater, C.W., and W.R. Fehr, Association of Total Sugar Content with Other Seed Traits of Diverse Soybean Cultivars, *Crop Sci.* 40:1552–1555 (2000).
185. Main, E.L., D.M. Pharr, S.C. Huber, and D.E. Moreland, Control of Galactosyl-Sugar Metabolism in Relation to Rate of Germination, *Physiol. Plant* 59:387–392 (1983).
186. Ohlrogge, J., and J.G. Jaworski, Regulation of Fatty Acid Synthesis, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:109–136 (1997).
187. Hitz, W.D., N.S. Yadav, R.S. Reiter, C.J. Mauvais, and A.J. Kinney, Reducing Polyunsaturation in Oils of Transgenic Canola and Soybean, in *Plant Lipid Metabolism*, edited by J.-C. Kader *et al.*, Kluwer Academic Publishers, Dordrecht, Netherlands, 1995, pp. 506–508.
188. Shanklin, J., E.B. Cahoon, E. Whittle, Y. Lindqvist, W. Huang, G. Schneider, and H. Schmidt, Structure-Function Studies on Desaturases and Related Hydrocarbon Hydroxylases, in *Physiology, Biochemistry, and Molecular Biology of Plant Lipids*, edited by J.P. Williams *et al.*, Kluwer Academic Publishers, Dordrecht, Netherlands, 1997, pp. 6–10.
189. Diers, B.W., and R.C. Shoemaker, Restriction Fragment Length Polymorphism Analysis of Soybean Fatty Acid Content, *J. Am. Oil Chem. Soc.* 69:1242–1244 (1992).
190. Li, Z., R.F. Wilson, W.E. Rayford, and H.R. Boerma, Molecular Mapping of Genes Controlling Reduced Palmitic Acid Content in Soybean, *Crop Sci.* 43:373–378 (2003).
191. Wilson, R.F., P. Kwanyuen, and J.W. Burton, Biochemical Characterization of a Genetic Trait for Low Palmitic Acid Content in Soybean, in *Proceedings of the World Conference on Biotechnology for the Fats and Oils Industry*, edited by T.H. Applewhite, AOCS Press, Champaign, Illinois, 1988, pp. 290–293.
192. Burton, J.W., R.F. Wilson, and C.A. Brim, Registration of N79-2077-12 and N87-2122-4, Two Soybean Germplasm Lines with Reduced Palmitic Acid in Seed Oil, *Crop Sci.* 34:313 (1994).
193. Erickson, E.A., J.R. Wilcox, and J.F. Cavins, Inheritance of Altered Palmitic Acid Percentage in Two Soybean Mutants, *J. Hered.* 465–468 (1988).
194. Schnebly, S.R., W.R. Fehr, G.A. Welke, E.G. Hammond, and D.N. Duvick, Inheritance of Reduced and Elevated Palmitate in Mutant Lines of Soybean, *Crop Sci.* 34:829–833 (1994).
195. Stojsin, D., G.R. Ablett, B.M. Luzzi, and J.W. Tanner, Use of Gene Substitution Values to Quantify Partial Dominance in Low Palmitic Acid Soybean, *Crop Sci.* 38:1437–1441 (1998).
196. Horejsi, T.F., W.R. Fehr, G.A. Welke, D.N. Duvick, E.G. Hammond, and S.R. Cianzio, Genetic Control of Reduced Palmitate Content in Soybean, *Crop Sci.* 34:331–334 (1994).
197. Wilcox, J.R., J.W. Burton, G.J. Rebetzke, and R.F. Wilson, Transgressive Segregation for Palmitic Acid in Seed Oil of Soybean, *Crop Sci.* 34:1248–1250 (1994).

198. Burton, J.W., J.R. Wilcox, R.F. Wilson, W.P. Novitzky, and G.J. Rebetzke, Registration of Low Palmitic Acid Soybean Germplasm Lines N94-2575 and C1943, *Crop Sci.* 38:1407 (1998).
199. Wilson, R.F., T.C. Marquardt, W.P. Novitzky, J.W. Burton, J.R. Wilcox, A.J. Kinney, and R.E. Dewey, Metabolic Mechanisms Associated with Alleles Governing the 16:0 Concentration of Soybean Oil, *J. Am. Oil Chem. Soc.* 78:335–340 (2001).
200. Wilson, R.F., Alternatives to Genetically-Modified Soybeans: The Better Bean Initiative, *Lipid Technol.* 11:107–110 (1999).
201. Miquel, M.F., and J.A. Browse, High-Oleate Oilseeds Fail to Develop at Low Temperature, *Plant Physiol.* 106:421–427 (1994).
202. Burton, J.W., R.F. Wilson, C.A. Brim, and R.W. Rinne, Registration of Soybean Germplasm Lines with Modified Fatty Acid Composition of Seed Oil, *Crop Sci.* 29:1583 (1989).
203. Wilcox, J.R., J.F. Cavins, and N.C. Nielsen, Genetic Alteration of Soybean Oil Composition by a Chemical Mutagen, *J. Am. Oil Chem. Soc.* 61:97–100 (1984).
204. Wilcox, J.R., and J.F. Cavins, Inheritance of Low Linolenic Acid Content of the Seed Oil of a Mutant in *Glycine max*, *Theor. Appl. Genet.* 71:74–78 (1985).
205. Hawkins, S.E., W.R. Fehr, and E.G. Hammond, Resource Allocation in Breeding for Fatty Acid Composition of Soybean Oil, *Crop Sci.* 23:900–904 (1983).
206. Fehr, W.R., G.A. Welke, E.G. Hammond, D.N. Duvick, and S.R. Cianzio, Inheritance of Reduced Linolenic Acid Content in Soybean Genotypes A16 and A17, *Crop Sci.* 32:903–906 (1992).
207. Howell, R.W., C.A. Brim, and R.W. Rinne, The Plant Geneticist's Contribution toward Changing Lipid and Amino Acid Composition of Soybeans, *J. Am. Oil Chem. Soc.* 49:30–32 (1972).
208. Rennie, B.D., J. Zilka, M.M. Cramer, and W.D. Beversdorf, Genetic Analysis of Low Linolenic Acid Levels in the Soybean Line PI 361088B, *Crop Sci.* 28:655–657 (1988).
209. Rennie, B.D., and J.W. Tanner, Genetic Analysis of Low Linolenic Acid Levels in the Line PI 123440, *Soybean Genet. Newsl.* 16:25–26 (1989).
210. Gutfinger, T., and A. Letan. Studies of Unsaponifiables in Several Vegetable Oils. *Lipids* 9:658–663 (1974).
211. Sherwin, E.R., Antioxidants for Vegetable Oils, *J. Am. Oil Chem. Soc.* 53:430–436 (1976).
212. Mounts, T.L., K. Warner, G.R. List, W.E. Neff, and R.F. Wilson, Low-Linolenic Acid Soybean Oils—Alternatives to Frying Oils, *J. Am. Oil Chem. Soc.* 71:495–499 (1994).
213. Mounts, T.L., S.L. Abidi, and K.A. Rennick. Effect of Genetic Modification on the Content and Composition of Bioactive Constituents in Soybean Oil, *J. Am. Oil Chem. Soc.* 73:581–586 (1996).
214. Almonor, G.O., G.P. Fenner, and R.F. Wilson, Temperature Effects on Tocopherol Composition in Soybeans with Genetically Improved Oil Quality, *J. Am. Oil Chem. Soc.* 75:591–596 (1998).